Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
ethinyl estradiol and norgestrel covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein ethinyl estradiol and norgestrel are covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ethinyl estradiol and norgestrel are conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ethinyl estradiol and norgestrel from said composition in a pH-dependent manner.

19. A method for protecting ethinyl estradiol and norgestrel from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ethinyl estradiol and norgestrel from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ethinyl estradiol and norgestrel to said polypeptide.

21. A method for delivering ethinyl estradiol and norgestrel to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

ethinyl estradiol and norgestrel covalently attached to said polypeptide.

22. The method of claim 21 wherein ethinyl estradiol and norgestrel are released from said composition by an enzyme-catalyzed release.

CW164P

23. The method of claim 21 wherein ethinyl estradiol and norgestrel are released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW164P

Abstract

A composition comprising a polypeptide and ethinyl estradiol and norgestrel covalently attached to the polypeptide. Also provided is a method for delivery of ethinyl estradiol and norgestrel to a patient comprising administering to the patient a composition comprising a polypeptide and ethinyl estradiol and norgestrel covalently attached to the polypeptide. Also provided is a method for protecting ethinyl estradiol and norgestrel from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ethinyl estradiol and norgestrel from a composition comprising covalently attaching it to the polypeptide.

CW169P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING EXENDIN-4 AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to exendin-4, as well as methods for protecting and administering exendin-4. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Exendin-4 is a known pharmaceutical agent that is used in the treatment of diabetes. It is a synthetic form of a peptide isolated from the salivary secretions of the Gila monster lizard.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified, when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (exendin-4) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching exendin-4 to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising exendin-4 microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and exendin-4 covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Exendin-4 preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting exendin-4 from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering exendin-4 to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, exendin-4 is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, exendin-4 is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and exendin-4 is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, exendin-4 is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, exendin-4 is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching exendin-4 to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, exendin-4 and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize exendin-4 and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of exendin-4. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises exendin-4 covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, exendin-4 is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-exendin-4 conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW169P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
exendin-4 covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein exendin-4 is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein exendin-4 is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing exendin-4 from said composition in a pH-dependent manner.

19. A method for protecting exendin-4 from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of exendin-4 from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching exendin-4 to said polypeptide.

21. A method for delivering exendin-4 to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

exendin-4 covalently attached to said polypeptide.

22. The method of claim 21 wherein exendin-4 is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein exendin-4 is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW169P

Abstract

A composition comprising a polypeptide and exendin-4 covalently attached to the polypeptide. Also provided is a method for delivery of exendin-4 to a patient comprising administering to the patient a composition comprising a polypeptide and exendin-4 covalently attached to the polypeptide. Also provided is a method for protecting exendin-4 from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of exendin-4 from a composition comprising covalently attaching it to the polypeptide.

CW170P

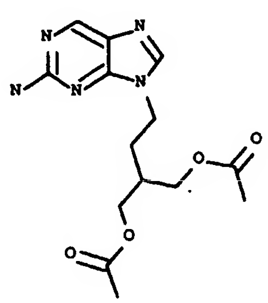
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FAMCICLOVIR AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to famciclovir, as well as methods for protecting and administering famciclovir. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Famciclovir is a known pharmaceutical agent that is used in the treatment of viral infection. Its chemical name is 2-[2-(2-amino-9H-purin-9-yl)ethyl]-1,3-propanediol diacetate. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (famciclovir) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching famciclovir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising famciclovir microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and famciclovir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Famciclovir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting famciclovir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering famciclovir to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, famciclovir is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, famciclovir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and famciclovir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, famciclovir is

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, famciclovir is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching famciclovir to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, famciclovir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize famciclovir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of famciclovir. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Famciclovir is the subject of EP 182024 B (1991) and U.S. Patent Number 5,246,937, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises famciclovir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, famciclovir is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-famciclovir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
famciclovir covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein famciclovir is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein famciclovir is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing famciclovir from said composition in a pH-dependent manner.

19. A method for protecting famciclovir from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of famciclovir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching famciclovir to said polypeptide.

21. A method for delivering famciclovir to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

famciclovir covalently attached to said polypeptide.

22. The method of claim 21 wherein famciclovir is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein famciclovir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and famciclovir covalently attached to the polypeptide. Also provided is a method for delivery of famciclovir to a patient comprising administering to the patient a composition comprising a polypeptide and famciclovir covalently attached to the polypeptide. Also provided is a method for protecting famciclovir from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of famciclovir from a composition comprising covalently attaching it to the polypeptide.

CW171P

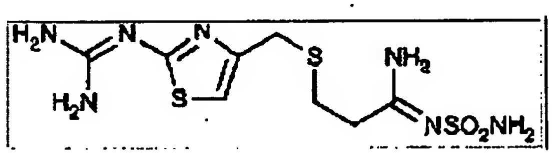
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FAMOTIDINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to famotidine, as well as methods for protecting and administering famotidine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Famotidine is a known pharmaceutical agent that is used in the treatment of ulcers and heartburn. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (famotidine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching famotidine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising famotidine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and famotidine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Famotidine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting famotidine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering famotidine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, famotidine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, famotidine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and famotidine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, famotidine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, famotidine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the

composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching famotidine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, famotidine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize famotidine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of famotidine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises famotidine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, famotidine is covalently attached to the polypeptide via the amino groups.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-famotidine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

famotidine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein famotidine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein famotidine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing famotidine from said composition in a pH-dependent manner.

19. A method for protecting famotidine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of famotidine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching famotidine to said polypeptide.

21. A method for delivering famotidine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

famotidine covalently attached to said polypeptide.

22. The method of claim 21 wherein famotidine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein famotidine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW171P

Abstract

A composition comprising a polypeptide and famotidine covalently attached to the polypeptide. Also-provided is a method for delivery of famotidine to a patient comprising administering to the patient a composition comprising a polypeptide and famotidine covalently attached to the polypeptide. Also provided is a method for protecting famotidine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of famotidine from a composition comprising covalently attaching it to the polypeptide.

CW172P

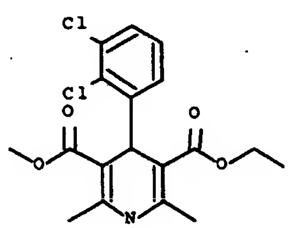
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FELODIPINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to felodipine, as well as methods for protecting and administering felodipine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Felodipine is a known pharmaceutical agent that is used in the treatment of hypertension. Its chemical name is 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylic acid ethyl methyl ester. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (felodipine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching felodipine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising felodipine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and felodipine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Felodipine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting felodipine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering felodipine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, felodipine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, felodipine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and felodipine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, felodipine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, felodipine is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching felodipine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, felodipine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize felodipine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of felodipine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Felodipine is the subject of U.S. Patent Numbers 4,264,611 and 4,803,081, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises felodipine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-felodipine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
felodipine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein felodipine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein felodipine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing felodipine from said composition in a pH-dependent manner.

19. A method for protecting felodipine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of felodipine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching felodipine to said polypeptide.

21. A method for delivering felodipine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

felodipine covalently attached to said polypeptide.

22. The method of claim 21 wherein felodipine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein felodipine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW172P

Abstract

A composition comprising a polypeptide and felodipine covalently attached to the polypeptide. Also provided is a method for delivery of felodipine to a patient comprising administering to the patient a composition comprising a polypeptide and felodipine covalently attached to the polypeptide. Also provided is a method for protecting felodipine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of felodipine from a composition comprising covalently attaching it to the polypeptide.

CW173P

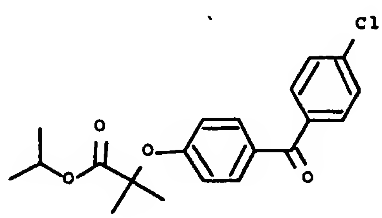
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FENOFIBRATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fenofibrate, as well as methods for protecting and administering fenofibrate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fenofibrate is a known pharmaceutical agent that is used in the treatment of hyperlipiemia. Its chemical name is 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoic acid 1-methylethyl ester. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fenofibrate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fenofibrate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fenofibrate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fenofibrate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fenofibrate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fenofibrate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fenofibrate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fenofibrate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fenofibrate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fenofibrate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fenofibrate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fenofibrate is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fenofibrate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fenofibrate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fenofibrate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fenofibrate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fenofibrate is the subject of U.S. Patent Number 4,895,726, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fenofibrate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fenofibrate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fenofibrate covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fenofibrate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fenofibrate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fenofibrate from said composition in a pH-dependent manner.

19. A method for protecting fenofibrate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fenofibrate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fenofibrate to said polypeptide.

21. A method for delivering fenofibrate to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fenofibrate covalently attached to said polypeptide.

22. The method of claim 21 wherein fenofibrate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fenofibrate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and fenofibrate covalently attached to the polypeptide. Also provided is a method for delivery of fenofibrate to a patient comprising administering to the patient a composition comprising a polypeptide and fenofibrate covalently attached to the polypeptide. Also provided is a method for protecting fenofibrate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fenofibrate from a composition comprising covalently attaching it to the polypeptide.

CW174P

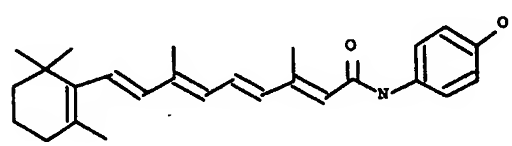
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FENRETINIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fenretinide, as well as methods for protecting and administering fenretinide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fenretinide is a known pharmaceutical agent that is used in the treatment of cancer. Its chemical name is N-(4-hydroxyphenyl)retinamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fenretinide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fenretinide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fenretinide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fenretinide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fenretinide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fenretinide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fenretinide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fenretinide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fenretinide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fenretinide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fenretinide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fenretinide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the

composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fenretinide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fenretinide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fenretinide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fenretinide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fenretinide is the subject of GB 1543824 (1979) – based on priority US application 628177 (1975), US 4,323,581 (1982), and US 4,665,098 (1987), , herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fenretinide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fenretinide is covalently attached to the polypeptide via the hydroxyl.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fenretinide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fenretinide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fenretinide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fenretinide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fenretinide from said composition in a pH-dependent manner.

19. A method for protecting fenretinide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fenretinide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fenretinide to said polypeptide.

21. A method for delivering fenretinide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fenretinide covalently attached to said polypeptide.

22. The method of claim 21 wherein fenretinide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fenretinide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW174P

Abstract

A composition comprising a polypeptide and fenretinide covalently attached to the polypeptide. Also provided is a method for delivery of fenretinide to a patient comprising administering to the patient a composition comprising a polypeptide and fenretinide covalently attached to the polypeptide. Also provided is a method for protecting fenretinide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fenretinide from a composition comprising covalently attaching it to the polypeptide.

CW175P

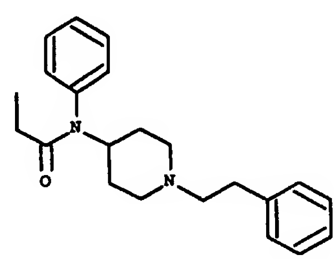
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FENTANYL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fentanyl, as well as methods for protecting and administering fentanyl. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fentanyl is a known pharmaceutical agent that is used in the treatment of pain. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. . Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fentanyl) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fentanyl to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fentanyl microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fentanyl covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fentanyl preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fentanyl from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fentanyl to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fentanyl is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fentanyl is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fentanyl is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fentanyl is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fentanyl is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fentanyl to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fentanyl and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fentanyl and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fentanyl. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises fentanyl covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fentanyl conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fentanyl covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fentanyl is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fentanyl is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fentanyl from said composition in a pH-dependent manner.

19. A method for protecting fentanyl from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fentanyl from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fentanyl to said polypeptide.

21. A method for delivering fentanyl to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fentanyl covalently attached to said polypeptide.

22. The method of claim 21 wherein fentanyl is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fentanyl is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW175P

Abstract

A composition comprising a polypeptide and fentanyl covalently attached to the polypeptide. Also provided is a method for delivery of fentanyl to a patient comprising administering to the patient a composition comprising a polypeptide and fentanyl covalently attached to the polypeptide. Also provided is a method for protecting fentanyl from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fentanyl from a composition comprising covalently attaching it to the polypeptide.

CW176P

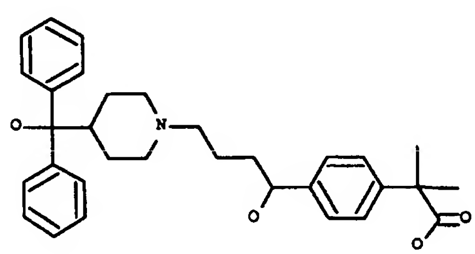
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FEXOFENADINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fexofenadine, as well as methods for protecting and administering fexofenadine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fexofenadine is a known pharmaceutical agent that is used in the treatment of seasonal allergic rhinitis. Its chemical name is 4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidinyl]butyl]-alpha,alpha-dimethylbenzeneacetic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fexofenadine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fexofenadine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fexofenadine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fexofenadine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fexofenadine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fexofenadine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fexofenadine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fexofenadine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fexofenadine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fexofenadine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment,

fexofenadine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fexofenadine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fexofenadine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fexofenadine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fexofenadine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fexofenadine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fexofenadine is the subject of U.S. Patent Numbers 4,254,129, 5,578,610, 5,855,912, 5,932,247, and 6,037,353, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fexofenadine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fexofenadine is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides.

Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fexofenadine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-

hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather,

various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW176P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fexofenadine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fexofenadine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fexofenadine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fexofenadine from said composition in a pH-dependent manner.

19. A method for protecting fexofenadine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fexofenadine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fexofenadine to said polypeptide.

21. A method for delivering fexofenadine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fexofenadine covalently attached to said polypeptide.

22. The method of claim 21 wherein fexofenadine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fexofenadine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and fexofenadine covalently attached to the polypeptide. Also provided is a method for delivery of fexofenadine to a patient comprising administering to the patient a composition comprising a polypeptide and fexofenadine covalently attached to the polypeptide. Also provided is a method for protecting fexofenadine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fexofenadine from a composition comprising covalently attaching it to the polypeptide.

CW177P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FILGRASTIM AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to filgrastim, as well as methods for protecting and administering filgrastim. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Filgrastim is a known pharmaceutical agent that is used in the treatment of cancer, HIV infection, pneumonia, leukopenia and skin ulcer. Its chemical name is N-L-methionyl-colony-stimulating factor (human clone 1034).

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (filgrastim) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching filgrastim to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising filgrastim microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and filgrastim covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Filgrastim preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting filgrastim from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering filgrastim to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, filgrastim is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, filgrastim is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and filgrastim is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, filgrastim is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, filgrastim is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching filgrastim to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, filgrastim and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize filgrastim and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of filgrastim. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Filgrastim is the subject of EP 237545 B (1991), based on priority application US 768959 (1985), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises filgrastim covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, filgrastim is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-filgrastim conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
filgrastim covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein filgrastim is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein filgrastim is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing filgrastim from said composition in a pH-dependent manner.

19. A method for protecting filgrastim from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of filgrastim from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching filgrastim to said polypeptide.

21. A method for delivering filgrastim to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

filgrastim covalently attached to said polypeptide.

22. The method of claim 21 wherein filgrastim is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein filgrastim is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW177P

Abstract

A composition comprising a polypeptide and filgrastim covalently attached to the polypeptide. Also provided is a method for delivery of filgrastim to a patient comprising administering to the patient a composition comprising a polypeptide and filgrastim covalently attached to the polypeptide. Also provided is a method for protecting filgrastim from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of filgrastim from a composition comprising covalently attaching it to the polypeptide.

CW178P

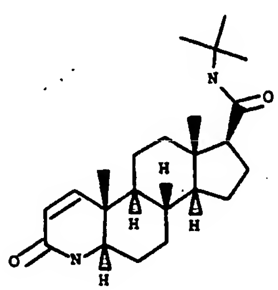
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FINASTERIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to finasteride, as well as methods for protecting and administering finasteride. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Finasteride is a known pharmaceutical agent that is used in the treatment of cancer, benign prostate hypertrophy, alopecia and acne. Its chemical name is (5alpha,17beta)-N-(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carb oxamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (finasteride) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching finasteride to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising finasteride microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and finasteride covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Finasteride preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting finasteride from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering finasteride to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, finasteride is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, finasteride is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and finasteride is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, finasteride is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, finasteride is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching finasteride to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, finasteride and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize finasteride and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of finasteride. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Finasteride is the subject of U.S. Patent Numbers 5,377,584, 4,760,071, 5,547,957, 5,571,817 and 5,886,184, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises finasteride covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, finasteride is covalently attached to the polypeptide via an amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-finasteride conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

finasteride covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein finasteride is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein finasteride is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing finasteride from said composition in a pH-dependent manner.

19. A method for protecting finasteride from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of finasteride from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching finasteride to said polypeptide.

21. A method for delivering finasteride to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

finasteride covalently attached to said polypeptide.

22. The method of claim 21 wherein finasteride is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein finasteride is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW178P

Abstract

A composition comprising a polypeptide and finasteride covalently attached to the polypeptide. Also provided is a method for delivery of finasteride to a patient comprising administering to the patient a composition comprising a polypeptide and finasteride covalently attached to the polypeptide. Also provided is a method for protecting finasteride from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of finasteride from a composition comprising covalently attaching it to the polypeptide.

CW179P

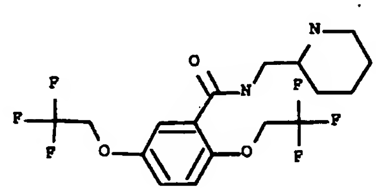
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLECAINIDE ACETATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to flecainide acetate, as well as methods for protecting and administering flecainide acetate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Flecainide acetate is a known pharmaceutical agent that is used in the treatment of arrythmia. Its chemical name is N-(2-piperidinylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (flecainide acetate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching flecainide acetate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising flecainide acetate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and flecainide acetate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Flecainide acetate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting flecainide acetate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering flecainide acetate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, flecainide acetate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, flecainide acetate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and flecainide acetate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, flecainide acetate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, flecainide acetate is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching flecainide acetate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, flecainide acetate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize flecainide acetate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of flecainide acetate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Flecainide acetate is the subject of U.S. Patent Number 4,642,384, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises flecainide acetate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide.. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, flecainide acetate is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-flecainide acetate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
flecainide acetate covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein flecainide acetate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein flecainide acetate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing flecainide acetate from said composition in a pH-dependent manner.

19. A method for protecting flecainide acetate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of flecainide acetate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching flecainide acetate to said polypeptide.

21. A method for delivering flecainide acetate to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

flecainide acetate covalently attached to said polypeptide.

22. The method of claim 21 wherein flecainide acetate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein flecainide acetate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW179P

Abstract

A composition comprising a polypeptide and flecainide acetate covalently attached to the polypeptide. Also provided is a method for delivery of flecainide acetate to a patient comprising administering to the patient a composition comprising a polypeptide and flecainide acetate covalently attached to the polypeptide. Also provided is a method for protecting flecainide acetate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of flecainide acetate from a composition comprising covalently attaching it to the polypeptide.

CW180P

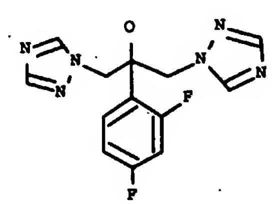
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUCONAZOLE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fluconazole, as well as methods for protecting and administering fluconazole. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fluconazole is a known pharmaceutical agent that is used in the treatment of fungal infections. Its chemical name is alpha-(2,4-difluorophenyl)-alpha-(1H-1,2,4-triazol-1-ylmethyl)-1H-1,2,4-triazole-1-ethanol. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fluconazole) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fluconazole to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fluconazole microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fluconazole covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fluconazole preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fluconazole from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fluconazole to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fluconazole is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fluconazole is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fluconazole is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fluconazole is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fluconazole is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fluconazole to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fluconazole and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fluconazole and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fluconazole. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fluconazole is the subject of U.S. Patent Numbers 4,404,216 and 4,416,682, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fluconazole covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fluconazole is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fluconazole conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fluconazole covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fluconazole is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fluconazole is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fluconazole from said composition in a pH-dependent manner.

19. A method for protecting fluconazole from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fluconazole from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fluconazole to said polypeptide.

21. A method for delivering fluconazole to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fluconazole covalently attached to said polypeptide.

22. The method of claim 21 wherein fluconazole is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fluconazole is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW180P

Abstract

A composition comprising a polypeptide and fluconazole covalently attached to the polypeptide. Also provided is a method for delivery of fluconazole to a patient comprising administering to the patient a composition comprising a polypeptide and fluconazole covalently attached to the polypeptide. Also provided is a method for protecting fluconazole from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fluconazole from a composition comprising covalently attaching it to the polypeptide.

CW181P

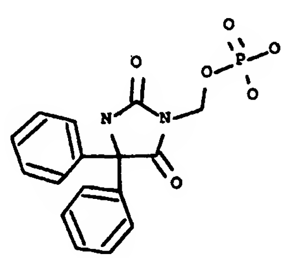
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUDROCORTISONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fludrocortisone, as well as methods for protecting and administering fludrocortisone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fludrocortisone is a known pharmaceutical agent that is used in the treatment of epilepsy. Its chemical name is 5,5-diphenyl-3-[(phosphonooxy)methyl]-2,4-imidazolidinedion. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fludrocortisone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fludrocortisone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fludrocortisone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fludrocortisone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fludrocortisone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fludrocortisone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fludrocortisone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fludrocortisone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fludrocortisone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fludrocortisone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fludrocortisone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fludrocortisone is

released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fludrocortisone to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fludrocortisone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fludrocortisone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fludrocortisone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fludrocortisone is the subject of U.S. Patent Number 4,260,769 (1981), and EP 473687 B (1996), based on priority application US 356948 (1989), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fludrocortisone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine.. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fludrocortisone is covalently attached to the polypeptide via the phosphate group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fludrocortisone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW181P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fludrocortisone covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fludrocortisone is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fludrocortisone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fludrocortisone from said composition in a pH-dependent manner.

19. A method for protecting fludrocortisone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fludrocortisone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fludrocortisone to said polypeptide.

21. A method for delivering fludrocortisone to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fludrocortisone covalently attached to said polypeptide.

22. The method of claim 21 wherein fludrocortisone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fludrocortisone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and fludrocortisone covalently attached to the polypeptide. Also provided is a method for delivery of fludrocortisone to a patient comprising administering to the patient a composition comprising a polypeptide and fludrocortisone covalently attached to the polypeptide. Also provided is a method for protecting fludrocortisone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fludrocortisone from a composition comprising covalently attaching it to the polypeptide.

CW182P

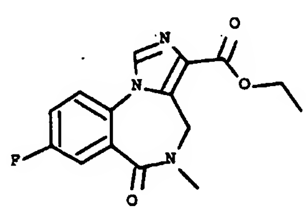
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUMAZENIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to flumazenil, as well as methods for protecting and administering flumazenil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Flumazenil is a known pharmaceutical agent that is used in the treatment of depression and liver disease. Its chemical name is 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylic acid ethyl ester. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (flumazenil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching flumazenil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising flumazenil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and flumazenil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Flumazenil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting flumazenil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering flumazenil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, flumazenil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, flumazenil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and flumazenil is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, flumazenil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, flumazenil is released from the composition in a sustained

release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching flumazenil to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, flumazenil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize flumazenil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of flumazenil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Flumazenil is the subject of U.S. Patent Number 4,316,839, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises flumazenil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
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Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythireonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-flumazenil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
flumazenil covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein flumazenil is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein flumazenil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing flumazenil from said composition in a pH-dependent manner.

19. A method for protecting flumazenil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of flumazenil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching flumazenil to said polypeptide.

21. A method for delivering flumazenil to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

flumazenil covalently attached to said polypeptide.

22. The method of claim 21 wherein flumazenil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein flumazenil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and flumazenil covalently attached to the polypeptide. Also provided is a method for delivery of flumazenil to a patient comprising administering to the patient a composition comprising a polypeptide and flumazenil covalently attached to the polypeptide. Also provided is a method for protecting flumazenil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of flumazenil from a composition comprising covalently attaching it to the polypeptide.

CW183P

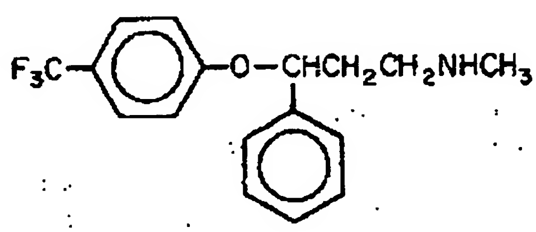
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUOXETINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fluoxetine, as well as methods for protecting and administering fluoxetine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fluoxetine is a known pharmaceutical agent that is used in the treatment of depression. Its chemical name is (N-methyl 3-(p-trifluoromethylphenoxy)-3-phenylpropylamine. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fluoxetine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fluoxetine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fluoxetine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fluoxetine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fluoxetine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fluoxetine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fluoxetine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fluoxetine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fluoxetine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fluoxetine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fluoxetine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fluoxetine is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fluoxetine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fluoxetine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fluoxetine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fluoxetine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fluoxetine is the subject of U.S. Patent Number 4,329,356, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fluoxetine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fluoxetine is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fluoxetine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fluoxetine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fluoxetine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fluoxetine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fluoxetine from said composition in a pH-dependent manner.

19. A method for protecting fluoxetine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fluoxetine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fluoxetine to said polypeptide.

21. A method for delivering fluoxetine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fluoxetine covalently attached to said polypeptide.

22. The method of claim 21 wherein fluoxetine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fluoxetine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and fluoxetine covalently attached to the polypeptide. Also provided is a method for delivery of fluoxetine to a patient comprising administering to the patient a composition comprising a polypeptide and fluoxetine covalently attached to the polypeptide. Also provided is a method for protecting fluoxetine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fluoxetine from a composition comprising covalently attaching it to the polypeptide.

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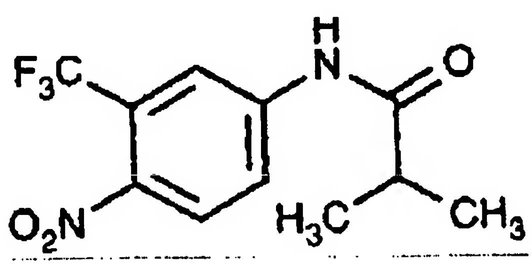
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUTAMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to flutamide, as well as methods for protecting and administering flutamide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Flutamide is a known pharmaceutical agent that is used in the treatment of prostate cancer. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (flutamide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching flutamide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising flutamide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and flutamide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Flutamide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting flutamide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering flutamide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, flutamide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, flutamide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and flutamide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, flutamide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, flutamide is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching flutamide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, flutamide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize flutamide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of flutamide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises flutamide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, flutamide is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-flutamide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
flutamide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein flutamide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein flutamide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing flutamide from said composition in a pH-dependent manner.

19. A method for protecting flutamide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of flutamide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching flutamide to said polypeptide.

21. A method for delivering flutamide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

flutamide covalently attached to said polypeptide.

22. The method of claim 21 wherein flutamide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein flutamide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW184P

Abstract

A composition comprising a polypeptide and flutamide covalently attached to the polypeptide. Also provided is a method for delivery of flutamide to a patient comprising administering to the patient a composition comprising a polypeptide and flutamide covalently attached to the polypeptide. Also provided is a method for protecting flutamide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of flutamide from a composition comprising covalently attaching it to the polypeptide.

CW185P

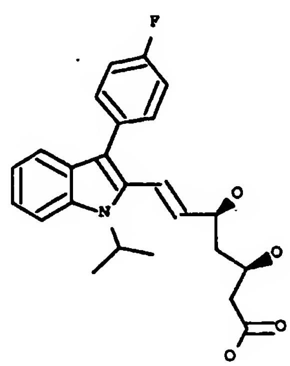
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUVASTATIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fluvastatin, as well as methods for protecting and administering fluvastatin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fluvastatin is a known pharmaceutical agent that is used in the treatment of hyperlipidemia. Its chemical name is (3R,5S,6E)-rel-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2-yl]-3,5-dihydroxy-6-heptenoic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fluvastatin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fluvastatin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fluvastatin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fluvastatin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fluvastatin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fluvastatin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fluvastatin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fluvastatin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fluvastatin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fluvastatin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fluvastatin is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fluvastatin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fluvastatin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fluvastatin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fluvastatin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fluvastatin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fluvastatin is the subject of U.S. Patent Number 5,354,772, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fluvastatin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fluvastatin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fluvastatin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine. .

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fluvastatin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fluvastatin from said composition in a pH-dependent manner.

19. A method for protecting fluvastatin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fluvastatin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fluvastatin to said polypeptide.

21. A method for delivering fluvastatin to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fluvastatin covalently attached to said polypeptide.

22. The method of claim 21 wherein fluvastatin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fluvastatin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fluvastatin covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fluvastatin is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

Abstract

A composition comprising a polypeptide and fluvastatin covalently attached to the polypeptide. Also provided is a method for delivery of fluvastatin to a patient comprising administering to the patient a composition comprising a polypeptide and fluvastatin covalently attached to the polypeptide. Also provided is a method for protecting fluvastatin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fluvastatin from a composition comprising covalently attaching it to the polypeptide.

CW186P

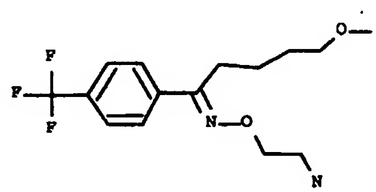
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FLUVOXAMINE MALEATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fluvoxamine maleate, as well as methods for protecting and administering fluvoxamine maleate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fluvoxamine maleate is a known pharmaceutical agent that is used in the treatment of depression and anxiety. Its chemical name is 5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone (E)-O-(2-aminoethyl)oxime. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fluvoxamine maleate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fluvoxamine maleate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fluvoxamine maleate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fluvoxamine maleate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fluvoxamine maleate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fluvoxamine maleate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fluvoxamine maleate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fluvoxamine maleate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fluvoxamine maleate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fluvoxamine maleate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fluvoxamine maleate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fluvoxamine

maleate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fluvoxamine maleate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fluvoxamine maleate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fluvoxamine maleate and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fluvoxamine maleate. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fluvoxamine maleate is the subject of GB1535226 (1978), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fluvoxamine maleate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fluvoxamine maleate is covalently attached to the polypeptide via the amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fluvoxamine maleate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fluvoxamine maleate covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fluvoxamine maleate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fluvoxamine maleate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fluvoxamine maleate from said composition in a pH-dependent manner.

19. A method for protecting fluvoxamine maleate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fluvoxamine maleate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fluvoxamine maleate to said polypeptide.

21. A method for delivering fluvoxamine maleate to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fluvoxamine maleate covalently attached to said polypeptide.

22. The method of claim 21 wherein fluvoxamine maleate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fluvoxamine maleate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW186P

Abstract

A composition comprising a polypeptide and fluvoxamine maleate covalently attached to the polypeptide. Also provided is a method for delivery of fluvoxamine maleate to a patient comprising administering to the patient a composition comprising a polypeptide and fluvoxamine maleate covalently attached to the polypeptide. Also provided is a method for protecting fluvoxamine maleate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fluvoxamine maleate from a composition comprising covalently attaching it to the polypeptide.

CW187P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FOLLITROPIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to follitropin, as well as methods for protecting and administering follitropin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Follitropin is a known pharmaceutical agent that is used in the treatment of infertility. Its chemical name is follicle-stimulating hormone (human alpha-subunit reduced), complex with follicle-stimulating hormone (human beta-subunit reduced).

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (follitropin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching follitropin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising follitropin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and follitropin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Follitropin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting follitropin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering follitropin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, follitropin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, follitropin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and follitropin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, follitropin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, follitropin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching follitropin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, follitropin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize follitropin and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of follitropin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Follitropin is the subject of WO 95/19991 (1995) and U.S. Patent Numbers 4,589,402, 5,270,057, and 5,767,251, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises follitropin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, follitropin is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-follitropin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
follitropin covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein follitropin is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein follitropin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing follitropin from said composition in a pH-dependent manner.

19. A method for protecting follitropin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of follitropin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching follitropin to said polypeptide.

21. A method for delivering follitropin to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

follitropin covalently attached to said polypeptide.

22. The method of claim 21 wherein follitropin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein follitropin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and follitropin covalently attached to the polypeptide. Also provided is a method for delivery of follitropin to a patient comprising administering to the patient a composition comprising a polypeptide and follitropin covalently attached to the polypeptide. Also provided is a method for protecting follitropin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of follitropin from a composition comprising covalently attaching it to the polypeptide.

CW188P

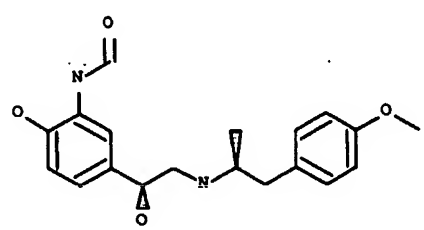
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FORMOTEROL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to formoterol, as well as methods for protecting and administering formoterol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Formoterol is a known pharmaceutical agent that is used in the treatment of asthma. Its chemical name is rel-N-[2-hydroxy-5-[(1R)-1-hydroxy-2-[[(1R)-2-(4-methoxypheny)-1-methylethyl]amino]ethyl]phenyl]formamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (formoterol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching formoterol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising formoterol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and formoterol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Formoterol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting formoterol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering formoterol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, formoterol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, formoterol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and formoterol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, formoterol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, formoterol is released from the composition in a sustained

release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching formoterol to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, formoterol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize formoterol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of formoterol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Formoterol is the subject of GB 1415256 (1975), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises formoterol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, formoterol is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-formoterol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

formoterol covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein formoterol is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein formoterol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing formoterol from said composition in a pH-dependent manner.

19. A method for protecting formoterol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of formoterol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching formoterol to said polypeptide.

21. A method for delivering formoterol to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

formoterol covalently attached to said polypeptide.

22. The method of claim 21 wherein formoterol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein formoterol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and formoterol covalently attached to the polypeptide. Also provided is a method for delivery of formoterol to a patient comprising administering to the patient a composition comprising a polypeptide and formoterol covalently attached to the polypeptide. Also provided is a method for protecting formoterol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of formoterol from a composition comprising covalently attaching it to the polypeptide.

CW189P

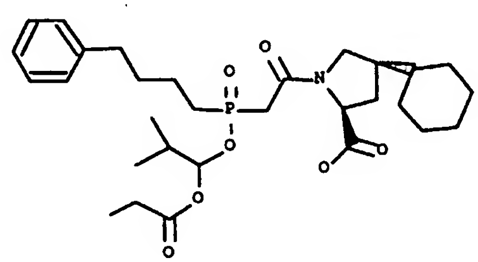
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FOSINOPRIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fosinopril, as well as methods for protecting and administering fosinopril. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fosinopril is a known pharmaceutical agent that is used in the treatment of hypertension. Its chemical name is [1[S*(R*)],2alpha.,4beta]-4-cyclohexyl-1-[[[2-methyl-1-(1-oxopropoxy)propoxy](4-phenylbutyl)phosphinyl]acetyl]-L-proline. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fosinopril) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fosinopril to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fosinopril microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fosinopril covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fosinopril preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fosinopril from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fosinopril to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fosinopril is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fosinopril is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fosinopril is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fosinopril is released

from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fosinopril is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fosinopril to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fosinopril and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention.

The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fosinopril and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fosinopril. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fosinopril is the subject of U.S. Patent Number 4,337,201, 4,384,123, and 5,006,344, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fosinopril covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fosinopril is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fosinopril conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fosinopril covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fosinopril is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fosinopril is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fosinopril from said composition in a pH-dependent manner.

19. A method for protecting fosinopril from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fosinopril from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fosinopril to said polypeptide.

21. A method for delivering fosinopril to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fosinopril covalently attached to said polypeptide.

22. The method of claim 21 wherein fosinopril is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fosinopril is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and fosinopril covalently attached to the polypeptide. Also provided is a method for delivery of fosinopril to a patient comprising administering to the patient a composition comprising a polypeptide and fosinopril covalently attached to the polypeptide. Also provided is a method for protecting fosinopril from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fosinopril from a composition comprising covalently attaching it to the polypeptide.

CW190P

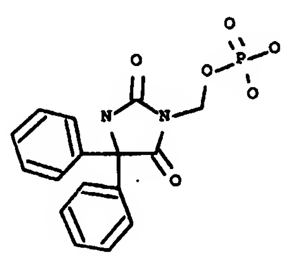
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FOSPHENYTOIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to fosphenytoin, as well as methods for protecting and administering fosphenytoin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Fosphenytoin is a known pharmaceutical agent that is used in the treatment of epilepsy. Its chemical name is 5,5-diphenyl-3-[(phosphonooxy)methyl]-2,4-imidazolidinedione. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (fosphenytoin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching fosphenytoin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising fosphenytoin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and fosphenytoin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Fosphenytoin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting fosphenytoin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering fosphenytoin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, fosphenytoin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, fosphenytoin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and fosphenytoin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, fosphenytoin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, fosphenytoin is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching fosphenytoin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, fosphenytoin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize fosphenytoin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of fosphenytoin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Fosphenytoin is the subject of U.S. Patent Numbers 4,260,769 and 4,925,860, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises fosphenytoin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, fosphenytoin is covalently attached to the polypeptide via the phosphate or amino group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-fosphenytoin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
fosphenytoin covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein fosphenytoin is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein fosphenytoin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing fosphenytoin from said composition in a pH-dependent manner.

19. A method for protecting fosphenytoin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of fosphenytoin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching fosphenytoin to said polypeptide.

21. A method for delivering fosphenytoin to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

fosphenytoin covalently attached to said polypeptide.

22. The method of claim 21 wherein fosphenytoin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein fosphenytoin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW190P

Abstract

A composition comprising a polypeptide and fosphenytoin covalently attached to the polypeptide. Also provided is a method for delivery of fosphenytoin to a patient comprising administering to the patient a composition comprising a polypeptide and fosphenytoin covalently attached to the polypeptide. Also provided is a method for protecting fosphenytoin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of fosphenytoin from a composition comprising covalently attaching it to the polypeptide.

CW191P

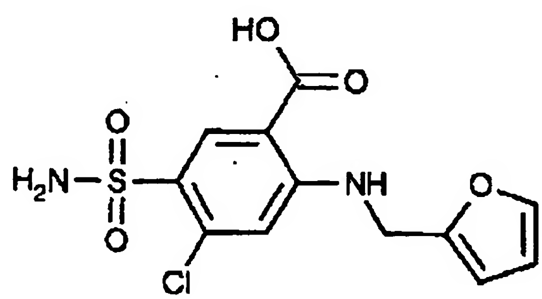
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING FUROSEMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to furosemide, as well as methods for protecting and administering furosemide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Furosemide is a known pharmaceutical agent that is used in the treatment of edema and hypertension. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (furosemide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching furosemide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising furosemide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and furosemide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Furosemide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting furosemide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering furosemide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, furosemide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, furosemide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and furosemide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, furosemide is

released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, furosemide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching furosemide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, furosemide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize furosemide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of furosemide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises furosemide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, furosemide is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-furosemide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW191P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
furosemide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein furosemide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein furosemide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing furosemide from said composition in a pH-dependent manner.

19. A method for protecting furosemide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of furosemide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching furosemide to said polypeptide.

21. A method for delivering furosemide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

furosemide covalently attached to said polypeptide.

22. The method of claim 21 wherein furosemide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein furosemide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and furosemide covalently attached to the polypeptide. Also provided is a method for delivery of furosemide to a patient comprising administering to the patient a composition comprising a polypeptide and furosemide covalently attached to the polypeptide. Also provided is a method for protecting furosemide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of furosemide from a composition comprising covalently attaching it to the polypeptide.

CW192P

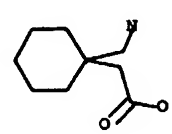
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GABAPENTIN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gabapentin, as well as methods for protecting and administering gabapentin. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gabapentin is a known pharmaceutical agent that is used in the treatment of epilepsy and depression. Its chemical name is 1-(aminomethyl)cyclohexaneacetic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken

under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent

in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR

application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gabapentin) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gabapentin to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gabapentin microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gabapentin covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gabapentin preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is

an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gabapentin from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gabapentin to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gabapentin is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gabapentin is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gabapentin is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gabapentin is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gabapentin is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be

microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gabapentin to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gabapentin and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gabapentin and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gabapentin. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gabapentin is the subject of U.S. Patent Numbers 4,087,544; 4,894,476; 5,084,479, and 6,054,482, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gabapentin covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the

protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gabapentin is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gabapentin conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can-then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW192P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gabapentin covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gabapentin is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gabapentin is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gabapentin from said composition in a pH-dependent manner.

19. A method for protecting gabapentin from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gabapentin from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gabapentin to said polypeptide.

21. A method for delivering gabapentin to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gabapentin covalently attached to said polypeptide.

22. The method of claim 21 wherein gabapentin is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gabapentin is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gabapentin covalently attached to the polypeptide. Also provided is a method for delivery of gabapentin to a patient comprising administering to the patient a composition comprising a polypeptide and gabapentin covalently attached to the polypeptide. Also provided is a method for protecting gabapentin from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gabapentin from a composition comprising covalently attaching it to the polypeptide.

CW193P

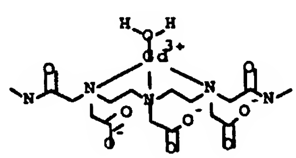
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GADODIAMIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gadodiamide, as well as methods for protecting and administering gadodiamide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gadodiamide is a known pharmaceutical agent that is used as a diagnostic agent in MRI. Its chemical name is [5,8-bis(carboxymethyl)-11-[2-(methylamino)-2-oxoethyl]-3-oxo- 2,5,8,11-tetraazatridecan-13-oato(3-)]gadolinium. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gadodiamide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gadodiamide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gadodiamide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gadodiamide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gadodiamide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gadodiamide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gadodiamide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gadodiamide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gadodiamide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gadodiamide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gadodiamide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gadodiamide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and

release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gadodiamide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gadodiamide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gadodiamide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gadodiamide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gadodiamide is the subject of U.S. Patent Number 4,687,659, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gadodiamide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gadodiamide is covalently attached to the polypeptide via the amino or hydroxy group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gadodiamide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gadodiamide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gadodiamide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gadodiamide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gadodiamide from said composition in a pH-dependent manner.

19. A method for protecting gadodiamide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gadodiamide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gadodiamide to said polypeptide.

21. A method for delivering gadodiamide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gadodiamide covalently attached to said polypeptide.

22. The method of claim 21 wherein gadodiamide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gadodiamide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW193P

Abstract

A composition comprising a polypeptide and gadodiamide covalently attached to the polypeptide. Also provided is a method for delivery of gadodiamide to a patient comprising administering to the patient a composition comprising a polypeptide and gadodiamide covalently attached to the polypeptide. Also provided is a method for protecting gadodiamide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gadodiamide from a composition comprising covalently attaching it to the polypeptide.

CW194P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GADOPENTETATE DIMEGLUMINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gadopentetate dimeglumine, as well as methods for protecting and administering gadopentetate dimeglumine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gadopentetate dimeglumine is a known pharmaceutical agent that is used for imaging in brain scans.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gadopentetate dimeglumine) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gadopentetate dimeglumine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gadopentetate dimeglumine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gadopentetate dimeglumine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gadopentetate dimeglumine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet

another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gadopentetate dimeglumine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gadopentetate dimeglumine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gadopentetate dimeglumine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gadopentetate dimeglumine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gadopentetate dimeglumine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gadopentetate dimeglumine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gadopentetate dimeglumine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The

adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gadopentetate dimeglumine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gadopentetate dimeglumine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gadopentetate dimeglumine and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gadopentetate dimeglumine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises gadopentetate dimeglumine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gadopentetate dimeglumine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be

stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gadopentetate dimeglumine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gadopentetate dimeglumine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gadopentetate dimeglumine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gadopentetate dimeglumine from said composition in a pH-dependent manner.

19. A method for protecting gadopentetate dimeglumine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gadopentetate dimeglumine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gadopentetate dimeglumine to said polypeptide.

21. A method for delivering gadopentetate dimeglumine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gadopentetate dimeglumine covalently attached to said polypeptide.

22. The method of claim 21 wherein gadopentetate dimeglumine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gadopentetate dimeglumine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gadopentetate dimeglumine covalently attached to the polypeptide. Also provided is a method for delivery of gadopentetate dimeglumine to a patient comprising administering to the patient a composition comprising a polypeptide and gadopentetate dimeglumine covalently attached to the polypeptide. Also provided is a method for protecting gadopentetate dimeglumine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gadopentetate dimeglumine from a composition comprising covalently attaching it to the polypeptide.

CW195P

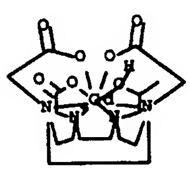
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GADOTERIDOL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gadoteridol, as well as methods for protecting and administering gadoteridol. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gadoteridol is a known pharmaceutical agent that is used as a contrast reagent in diagnostic imaging. Its chemical name is (+,-)-[10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7- triacetato(3-)]gadolinium. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gadoteridol) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gadoteridol to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gadoteridol microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gadoteridol covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gadoteridol preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gadoteridol from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gadoteridol to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gadoteridol is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gadoteridol is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gadoteridol is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gadoteridol is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gadoteridol is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant

from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gadoteridol to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gadoteridol and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gadoteridol and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gadoteridol. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gadoteridol is the subject of U.S. Patent Number 4,885,363, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gadoteridol covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gadoteridol is covalently attached to the polypeptide via a hydroxyl or amino group, or alternatively through an artificial linker.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gadoteridol conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0^oC. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0^oC. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gadoteridol covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gadoteridol is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gadoteridol is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gadoteridol from said composition in a pH-dependent manner.

19. A method for protecting gadoteridol from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gadoteridol from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gadoteridol to said polypeptide.

21. A method for delivering gadoteridol to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gadoteridol covalently attached to said polypeptide.

22. The method of claim 21 wherein gadoteridol is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gadoteridol is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW195P

Abstract

A composition comprising a polypeptide and gadoteridol covalently attached to the polypeptide. Also provided is a method for delivery of gadoteridol to a patient comprising administering to the patient a composition comprising a polypeptide and gadoteridol covalently attached to the polypeptide. Also provided is a method for protecting gadoteridol from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gadoteridol from a composition comprising covalently attaching it to the polypeptide.

CW196P

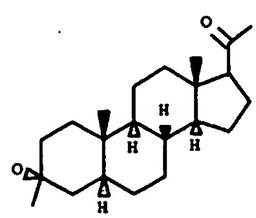
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GANAXOLONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ganaxolone, as well as methods for protecting and administering ganaxolone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ganaxolone is a known pharmaceutical agent that is used in the treatment of epilepsy and migraine. Its chemical name is (3alpha,5alpha)-3-hydroxy-3-methyl-pregnan-20-one. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ganaxolone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ganaxolone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ganaxolone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ganaxolone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ganaxolone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ganaxolone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ganaxolone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ganaxolone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, ganaxolone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ganaxolone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ganaxolone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ganaxolone is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching ganaxolone to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ganaxolone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ganaxolone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ganaxolone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Ganaxolone is the subject of DE 2162555 A (1972), WO 93/3732 (1993) – based on priority US application 745216 (1991), WO 93/5786 1993 – based on priority US application 759512 (1991), and WO 94/27608 (1994) – based on priority US application 68378 (1993), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises ganaxolone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding

are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ganaxolone is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ganaxolone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
ganaxolone covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein ganaxolone is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ganaxolone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ganaxolone from said composition in a pH-dependent manner.

19. A method for protecting ganaxolone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ganaxolone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ganaxolone to said polypeptide.

21. A method for delivering ganaxolone to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

ganaxolone covalently attached to said polypeptide.

22. The method of claim 21 wherein ganaxolone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ganaxolone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ganaxolone covalently attached to the polypeptide. Also provided is a method for delivery of ganaxolone to a patient comprising administering to the patient a composition comprising a polypeptide and ganaxolone covalently attached to the polypeptide. Also provided is a method for protecting ganaxolone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ganaxolone from a composition comprising covalently attaching it to the polypeptide.

CW197P

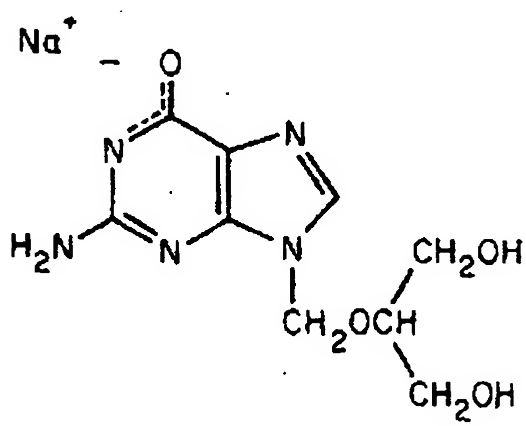
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GANCICLOVIR AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to ganciclovir, as well as methods for protecting and administering ganciclovir. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Ganciclovir is a known pharmaceutical agent that is used in the treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients, including patients with acquired immunodeficiency syndrome (AIDS). It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability

of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified

amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that

incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (ganciclovir) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching ganciclovir to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising ganciclovir microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and ganciclovir covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a

heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Ganciclovir preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting ganciclovir from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering ganciclovir to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, ganciclovir is released from the composition by

an enzyme-catalyzed release. In another preferred embodiment, ganciclovir is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and ganciclovir is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, ganciclovir is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, ganciclovir is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching ganciclovir to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, ganciclovir and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a

carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize ganciclovir and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of ganciclovir. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Ganciclovir is the subject of U.S. Patent Numbers 4,355,032, 4,423,050, 4,507,305 and 4,642,346, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises ganciclovir covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the

conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only

5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight

active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57.	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to

poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, ganciclovir is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the

mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-ganciclovir conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
ganciclovir covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein ganciclovir is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein ganciclovir is confotmationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing ganciclovir from said composition in a pH-dependent manner.

19. A method for protecting ganciclovir from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of ganciclovir from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching ganciclovir to said polypeptide.

21. A method for delivering ganciclovir to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

ganciclovir covalently attached to said polypeptide.

22. The method of claim 21 wherein ganciclovir is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein ganciclovir is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and ganciclovir covalently attached to the polypeptide. Also provided is a method for delivery of ganciclovir to a patient comprising administering to the patient a composition comprising a polypeptide and ganciclovir covalently attached to the polypeptide. Also provided is a method for protecting ganciclovir from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of ganciclovir from a composition comprising covalently attaching it to the polypeptide.

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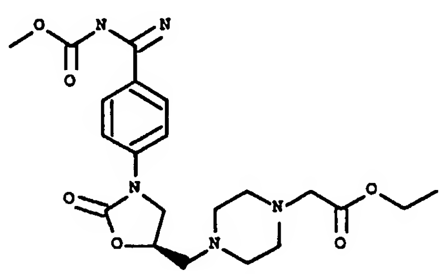
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GANTOFIBAN AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gantofiban, as well as methods for protecting and administering gantofiban. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gantofiban is a known pharmaceutical agent that is used in the treatment of thrombosis and angina. Its chemical name is 4-[[(5R)-3-[4-[imino[(methoxycarbonyl) amino]methyl]phenyl]-2-oxo-5-oxazolidinyl]methyl]-1-piperazineacetic acid ethyl ester 2-hydroxy-1,2,3-propanetricarboxylate (1:1. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical

compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several

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shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable

diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gantofiban) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gantofiban to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gantofiban microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gantofiban covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gastrin 17 immunogen from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gastrin 17 immunogen to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gastrin 17 immunogen is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gastrin 17 immunogen is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gastrin 17 immunogen is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gastrin 17 immunogen is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gastrin 17 immunogen is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gastrin 17 immunogen to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gastrin 17 immunogen and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gastrin 17 immunogen and prevent its digestion in the

stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gastrin 17 immunogen. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gastrin 17 immunogen is the subject of U.S. Patent Numbers 5622702, 5785970, 5607676, and 5609870, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gastrin 17 immunogen covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gastrin 17 immunogen is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gastrin 17 immunogen conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gastrin 17 immunogen covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gastrin 17 immunogen is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gastrin 17 immunogen is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gastrin 17 immunogen from said composition in a pH-dependent manner.

19. A method for protecting gastrin 17 immunogen from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gastrin 17 immunogen from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gastrin 17 immunogen to said polypeptide.

21. A method for delivering gastrin 17 immunogen to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gastrin 17 immunogen covalently attached to said polypeptide.

22. The method of claim 21 wherein gastrin 17 immunogen is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gastrin 17 immunogen is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gastrin 17 immunogen covalently attached to the polypeptide. Also provided is a method for delivery of gastrin 17 immunogen to a patient comprising administering to the patient a composition comprising a polypeptide and gastrin 17 immunogen covalently attached to the polypeptide. Also provided is a method for protecting gastrin 17 immunogen from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gastrin 17 immunogen from a composition comprising covalently attaching it to the polypeptide.

CW200P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GEMCITABINE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gemcitabine, as well as methods for protecting and administering gemcitabine. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gemcitabine is a known pharmaceutical agent that is used in the treatment of -------. Its chemical name is -----. Its structure is: -----

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another

invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (xxxxx) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gemcitabine to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gemcitabine microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gemcitabine covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gemcitabine preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gemcitabine from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gemcitabine to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gemcitabine is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gemcitabine is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gemcitabine is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gemcitabine is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gemcitabine is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gemcitabine to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gemcitabine and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gemcitabine and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of gemcitabine. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gemcitabine is the subject of U.S. Patent Number yyyyy, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gemcitabine covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
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Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gemcitabine is covalently attached to the polypeptide via the zzzzzzz.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gemcitabine conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gemcitabine covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gemcitabine is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gemcitabine is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gemcitabine from said composition in a pH-dependent manner.

19. A method for protecting gemcitabine from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gemcitabine from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gemcitabine to said polypeptide.

21. A method for delivering gemcitabine to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gemcitabine covalently attached to said polypeptide.

22. The method of claim 21 wherein gemcitabine is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gemcitabine is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW200P

Abstract

A composition comprising a polypeptide and gemcitabine covalently attached to the polypeptide. Also provided is a method for delivery of gemcitabine to a patient comprising administering to the patient a composition comprising a polypeptide and gemcitabine covalently attached to the polypeptide. Also provided is a method for protecting gemcitabine from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gemcitabine from a composition comprising covalently attaching it to the polypeptide.

CW201P

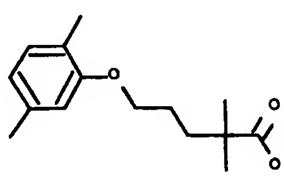
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GEMFIBROZIL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gemfibrozil, as well as methods for protecting and administering gemfibrozil. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gemfibrozil is a known pharmaceutical agent that is used in the treatment of hyperlipidemia. Its chemical name is 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gemfibrozil) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gemfibrozil to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gemfibrozil microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gemfibrozil covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gemfibrozil preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gemfibrozil from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gemfibrozil to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gemfibrozil is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gemfibrozil is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gemfibrozil is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gemfibrozil is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gemfibrozil is released from the

composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gemfibrozil to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gemfibrozil and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gemfibrozil and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gemfibrozil. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gemfibrozil is the subject of GB 1225575 (1971), priority based on US application 73046 (1968), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gemfibrozil covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a

particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the

carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular

weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-

carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gemfibrozil is covalently attached to the polypeptide via the carboxylic acid group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gemfibrozil conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0°C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gemfibrozil covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gemfibrozil is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gemfibrozil is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gemfibrozil from said composition in a pH-dependent manner.

19. A method for protecting gemfibrozil from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gemfibrozil from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gemfibrozil to said polypeptide.

21. A method for delivering gemfibrozil to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gemfibrozil covalently attached to said polypeptide.

22. The method of claim 21 wherein gemfibrozil is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gemfibrozil is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gemfibrozil covalently attached to the polypeptide. Also provided is a method for delivery of gemfibrozil to a patient comprising administering to the patient a composition comprising a polypeptide and gemfibrozil covalently attached to the polypeptide. Also provided is a method for protecting gemfibrozil from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gemfibrozil from a composition comprising covalently attaching it to the polypeptide.

CW202P

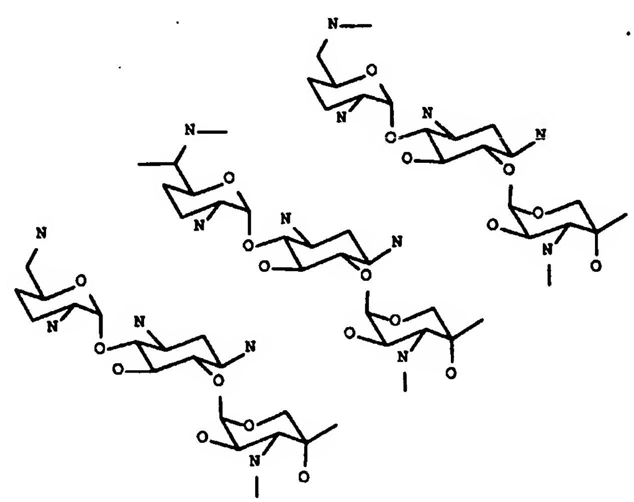
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GENTAMICIN ISOTON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gentamicin isoton, as well as methods for protecting and administering gentamicin isoton. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gentamicin isoton is a known pharmaceutical agent that is used in the treatment of bacterial infections and muscular dystrophy. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release

through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide

linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gentamicin isoton) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gentamicin isoton to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gentamicin isoton microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gentamicin isoton covalently attached to the polypeptide. Preferably, the polypeptide is (i) an

oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gentamicin isoton preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gentamicin isoton from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gentamicin isoton to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently

attached to the polypeptide. In a preferred embodiment, gentamicin isoton is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gentamicin isoton is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gentamicin isoton is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gentamicin isoton is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gentamicin isoton is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gentamicin isoton to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gentamicin isoton and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side

chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gentamicin isoton and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gentamicin isoton. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises gentamicin isoton covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes.

Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the

kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages: First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's

molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active

agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, gentamicin isoton is covalently attached to the polypeptide via the hydroxyl group.

The carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gentamicin isoton conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gentamicin isoton covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gentamicin isoton is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gentamicin isoton is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gentamicin isoton from said composition in a pH-dependent manner.

19. A method for protecting gentamicin isoton from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gentamicin isoton from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gentamicin isoton to said polypeptide.

21. A method for delivering gentamicin isoton to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gentamicin isoton covalently attached to said polypeptide.

22. The method of claim 21 wherein gentamicin isoton is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gentamicin isoton is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gentamicin isoton covalently attached to the polypeptide. Also provided is a method for delivery of gentamicin isoton to a patient comprising administering to the patient a composition comprising a polypeptide and gentamicin isoton covalently attached to the polypeptide. Also provided is a method for protecting gentamicin isoton from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gentamicin isoton from a composition comprising covalently attaching it to the polypeptide.

CW203P

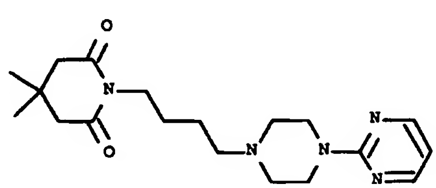
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GEPIRONE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to gepirone, as well as methods for protecting and administering gepirone. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Gepirone is a known pharmaceutical agent that is used in the treatment of anxiety and depression. Its chemical name is 4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (gepirone) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching gepirone to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising gepirone microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and gepirone covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Gepirone preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting gepirone from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering gepirone to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, gepirone is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, gepirone is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and gepirone is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, gepirone is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, gepirone is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching gepirone to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, gepirone and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize gepirone and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of gepirone. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Gepirone is the subject of GB 2114122 B (1985), based on priority US application 334688 (1981), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises gepirone covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, the active agent is covalently attached to the polypeptide via a linker. This linker may be a small molecule containing 2-6 carbons and one or more functional groups (such as amines, amides, alcohols, or acids) or may be made up of a short chain of either amino acids or carbohydrates.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-gepirone conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
gepirone covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein gepirone is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein gepirone is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing gepirone from said composition in a pH-dependent manner.

19. A method for protecting gepirone from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of gepirone from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching gepirone to said polypeptide.

21. A method for delivering gepirone to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

gepirone covalently attached to said polypeptide.

22. The method of claim 21 wherein gepirone is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein gepirone is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and gepirone covalently attached to the polypeptide. Also provided is a method for delivery of gepirone to a patient comprising administering to the patient a composition comprising a polypeptide and gepirone covalently attached to the polypeptide. Also provided is a method for protecting gepirone from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of gepirone from a composition comprising covalently attaching it to the polypeptide.

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A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GLATIRAMER ACETATE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to glatiramer acetate, as well as methods for protecting and administering glatiramer acetate. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Glatiramer acetate is a known pharmaceutical agent that is used in the treatment of multiple sclerosis. Its chemical name is L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (glatiramer acetate) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching glatiramer acetate to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising glatiramer acetate microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and glatiramer acetate covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Glatiramer acetate preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting glatiramer acetate from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering glatiramer acetate to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, glatiramer acetate is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, glatiramer acetate is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and glatiramer acetate is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, glatiramer acetate is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, glatiramer acetate is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching glatiramer acetate to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, glatiramer acetate and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize glatiramer acetate and prevent its digestion in the stomach. In

addition, the pharmacologic effect can be prolonged by delayed release of glatiramer acetate. Furthermore, active agents can be combined to produce synergistic effects.. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Glatiramer acetate is the subject of U.S. Patent Number 6,054,430 and 5,981,589, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises glatiramer acetate covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide.. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, glatiramer acetate is covalently attached to the polypeptide via a peptide bond.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-glatiramer acetate conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{\circ}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
glatiramer acetate covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein glatiramer acetate is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein glatiramer acetate is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing glatiramer acetate from said composition in a pH-dependent manner.

19. A method for protecting glatiramer acetate from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of glatiramer acetate from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching glatiramer acetate to said polypeptide.

21. A method for delivering glatiramer acetate to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

glatiramer acetate covalently attached to said polypeptide.

22. The method of claim 21 wherein glatiramer acetate is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein glatiramer acetate is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and glatiramer acetate covalently attached to the polypeptide. Also provided is a method for delivery of glatiramer acetate to a patient comprising administering to the patient a composition comprising a polypeptide and glatiramer acetate covalently attached to the polypeptide. Also provided is a method for protecting glatiramer acetate from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of glatiramer acetate from a composition comprising covalently attaching it to the polypeptide.

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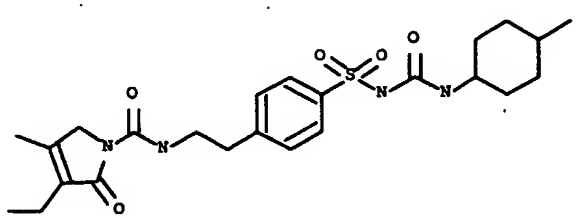
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GLIMEPIRIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to glimepiride, as well as methods for protecting and administering glimepiride. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Glimepiride is a known pharmaceutical agent that is used in the treatment of diabetes. Its chemical name is trans-3-ethyl-2,5-dihydro-4-methyl-N-[2-[4-[[[[(4-methylcyclohexyl)ami no]carbonyl]amino]sulfonyl]phenyl]ethyl]-2-oxo-1H-pyrrole-1-carboxamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (glimepiride) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching glimepiride to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising glimepiride microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and glimepiride covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Glimepiride preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting glimepiride from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering glimepiride to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, glimepiride is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, glimepiride is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and glimepiride is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, glimepiride is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, glimepiride is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching glimepiride to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, glimepiride and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize glimepiride and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of glimepiride. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Glimepiride is the subject of U.S. Patent Number 4,379,785, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises glimepiride covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, glimepiride is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-glimepiride conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
glimepiride covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein glimepiride is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein glimepiride is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing glimepiride from said composition in a pH-dependent manner.

19. A method for protecting glimepiride from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of glimepiride from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching glimepiride to said polypeptide.

21. A method for delivering glimepiride to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

glimepiride covalently attached to said polypeptide.

22. The method of claim 21 wherein glimepiride is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein glimepiride is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW205P

Abstract

A composition comprising a polypeptide and glimepiride covalently attached to the polypeptide. Also provided is a method for delivery of glimepiride to a patient comprising administering to the patient a composition comprising a polypeptide and glimepiride covalently attached to the polypeptide. Also provided is a method for protecting glimepiride from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of glimepiride from a composition comprising covalently attaching it to the polypeptide.

CW206P

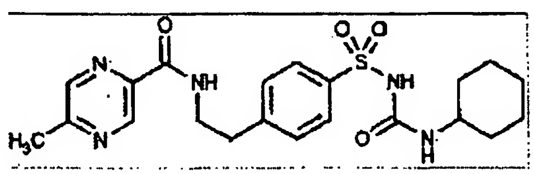
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GLIPIZIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to glipizide, as well as methods for protecting and administering glipizide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Glipizide is a known pharmaceutical agent that is used in the treatment of diabetes. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (glipizide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching glipizide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising glipizide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and glipizide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Glipizide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting glipizide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering glipizide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, glipizide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, glipizide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and glipizide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, glipizide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, glipizide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is

controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching glipizide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, glipizide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize glipizide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of glipizide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises glipizide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, glipizide is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-glipizide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
glipizide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein glipizide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein glipizide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing glipizide from said composition in a pH-dependent manner.

19. A method for protecting glipizide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of glipizide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching glipizide to said polypeptide.

21. A method for delivering glipizide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

glipizide covalently attached to said polypeptide.

22. The method of claim 21 wherein glipizide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein glipizide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW206P

Abstract

A composition comprising a polypeptide and glipizide covalently attached to the polypeptide. Also provided is a method for delivery of glipizide to a patient comprising administering to the patient a composition comprising a polypeptide and glipizide covalently attached to the polypeptide. Also provided is a method for protecting glipizide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of glipizide from a composition comprising covalently attaching it to the polypeptide.

CW207P

A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GLUCAGON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to glucagon, as well as methods for protecting and administering glucagon. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Glucagon is a known pharmaceutical agent that is used in the treatment of diabetes It is a naturally occurring peptide that can either be isolated or synthesized, preferably using recombinant DNA technology.

The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf

life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is

unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (glucagon) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching glucagon to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising glucagon microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and glucagon covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Glucagon preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tab'et, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting glucagon from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering glucagon to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, glucagon is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, glucagon is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and glucagon is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, glucagon is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, glucagon is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching glucagon to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, glucagon and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize glucagon and prevent its digestion in the stomach. In addition,

the pharmacologic effect can be prolonged by delayed release of glucagon. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises glucagon covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior

and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will

ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the

jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order

to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated,

preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, glucagon is covalently attached to the polypeptide via a peptide linkage.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known

intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-glucagon conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CW207P

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
glucagon covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein glucagon is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein glucagon is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing glucagon from said composition in a pH-dependent manner.

19. A method for protecting glucagon from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of glucagon from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching glucagon to said polypeptide.

21. A method for delivering glucagon to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

glucagon covalently attached to said polypeptide.

22. The method of claim 21 wherein glucagon is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein glucagon is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and glucagon covalently attached to the polypeptide. Also provided is a method for delivery of glucagon to a patient comprising administering to the patient a composition comprising a polypeptide and glucagon covalently attached to the polypeptide. Also provided is a method for protecting glucagon from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of glucagon from a composition comprising covalently attaching it to the polypeptide.

CW208P

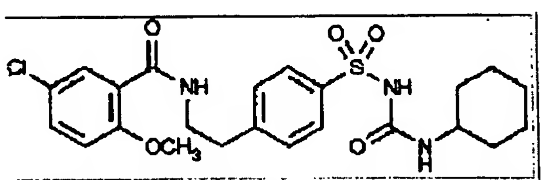
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GLYBURIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to glyburide, as well as methods for protecting and administering glyburide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Glyburide is a sulfonylurea antidiabetic agent used in the treatment of diabetes. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (glyburide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching glyburide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising glyburide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and glyburide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Glyburide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting glyburide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering glyburide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, glyburide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, glyburide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and glyburide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, glyburide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, glyburide is released from the composition in a sustained release.

In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching glyburide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, glyburide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize glyburide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of glyburide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises glyburide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, glyburide is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-glyburide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
glyburide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein glyburide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation..

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein glyburide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing glyburide from said composition in a pH-dependent manner.

19. A method for protecting glyburide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of glyburide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching glyburide to said polypeptide.

21. A method for delivering glyburide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

glyburide covalently attached to said polypeptide.

22. The method of claim 21 wherein glyburide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein glyburide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and glyburide covalently attached to the polypeptide. Also provided is a method for delivery of glyburide to a patient comprising administering to the patient a composition comprising a polypeptide and glyburide covalently attached to the polypeptide. Also provided is a method for protecting glyburide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of glyburide from a composition comprising covalently attaching it to the polypeptide.

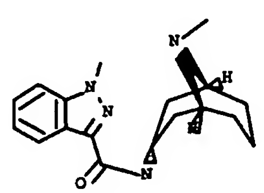
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING GRANISETRON AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to granisetron, as well as methods for protecting and administering granisetron. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Granisetron is a known pharmaceutical agent that is used in the treatment of nausea and vomiting in cancer patients Its chemical name is endo-1-methyl-N-(9-methyl-9-azabicyclo[3,3,1]non-3-yl)-1H-indazole-3-carboxamide. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (granisetron) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching granisetron to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising granisetron microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and granisetron covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Granisetron preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a

carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting granisetron from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering granisetron to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, granisetron is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, granisetron is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and granisetron is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, granisetron is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, granisetron is released from the composition in a

sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching granisetron to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, granisetron and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize granisetron and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of granisetron. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

Granisetron is the subject of U.S. Patent Number 4,886,808, herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises granisetron covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, granisetron is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-granisetron conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
granisetron covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein granisetron is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein granisetron is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing granisetron from said composition in a pH-dependent manner.

19. A method for protecting granisetron from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of granisetron from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching granisetron to said polypeptide.

21. A method for delivering granisetron to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

granisetron covalently attached to said polypeptide.

22. The method of claim 21 wherein granisetron is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein granisetron is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW209P

Abstract

A composition comprising a polypeptide and granisetron covalently attached to the polypeptide. Also provided is a method for delivery of granisetron to a patient comprising administering to the patient a composition comprising a polypeptide and granisetron covalently attached to the polypeptide. Also provided is a method for protecting granisetron from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of granisetron from a composition comprising covalently attaching it to the polypeptide.

CW210P

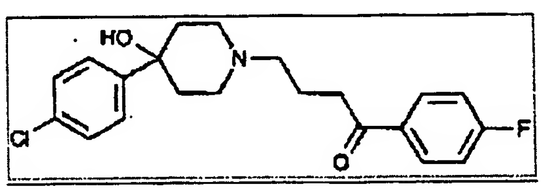
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING HALOPERIDAL AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to haloperidal, as well as methods for protecting and administering haloperidal. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Haloperidal is a known pharmaceutical agent that is used in the treatment of psychotic disorders. It is both commercially available and readily manufactured using published synthetic schemes by those of ordinary skill in the art. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these

systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble

microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients.

Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (haloperidal) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching haloperidal to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising haloperidal microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and haloperidal covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Haloperidal preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to

the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting haloperidal from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering haloperidal to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, haloperidal is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, haloperidal is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and haloperidal is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, haloperidal is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, haloperidal is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant

from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching haloperidal to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, haloperidal and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize haloperidal and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of haloperidal. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises haloperidal covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be

enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate

weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, haloperidal is covalently attached to the polypeptide via the hydroxyl group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-haloperidal conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to 0^{o}C. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product

precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for

several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to 0^oC. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
haloperidal covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein haloperidal is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein haloperidal is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing haloperidal from said composition in a pH-dependent manner.

19. A method for protecting haloperidal from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of haloperidal from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching haloperidal to said polypeptide.

21. A method for delivering haloperidal to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

haloperidal covalently attached to said polypeptide.

22. The method of claim 21 wherein haloperidal is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein haloperidal is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and haloperidal covalently attached to the polypeptide. Also provided is a method for delivery of haloperidal to a patient comprising administering to the patient a composition comprising a polypeptide and haloperidal covalently attached to the polypeptide. Also provided is a method for protecting haloperidal from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of haloperidal from a composition comprising covalently attaching it to the polypeptide.

CW211P

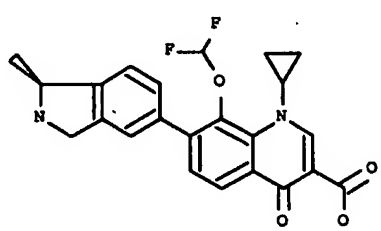
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to a quinolone antibiotic, as well as methods for protecting and administering a quinolone antibiotic. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

The quinolone antibiotic of the present invention is a known pharmaceutical agent that is used in the treatment of bacterial infections. Its chemical name is 1-cyclopropyl-8-(difluoromethoxy)-7-[(1R)-2,3-dihydro-1-methyl-1H-isoindol-5-yl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid monomethanesulfonate. Its structure is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. . Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (a quinolone antibiotic) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching a quinolone antibiotic to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising a quinolone antibiotic microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and a quinolone antibiotic covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

A quinolone antibiotic preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting a quinolone antibiotic from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering a quinolone antibiotic to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, a quinolone antibiotic is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, a quinolone antibiotic is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and a quinolone antibiotic is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, a quinolone antibiotic is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment, a quinolone

antibiotic is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching a quinolone antibiotic to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, a quinolone antibiotic and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize a quinolone antibiotic and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of a quinolone antibiotic. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

A quinolone antibiotic is the subject of EP 882725 A1 (1998), herein incorporated by reference, which describes how to make that drug.

The composition of the invention comprises a quinolone antibiotic covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino

acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's

decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of

active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent

delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using

any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence, maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, a quinolone antibiotic is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-a quinolone antibiotic conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{o}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

a polypeptide; and

a quinolone antibiotic covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein a quinolone antibiotic is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein a quinolone antibiotic is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing a quinolone antibiotic from said composition in a pH-dependent manner.

19. A method for protecting a quinolone antibiotic from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of a quinolone antibiotic from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching a quinolone antibiotic to said polypeptide.

21. A method for delivering a quinolone antibiotic to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

a quinolone antibiotic covalently attached to said polypeptide.

22. The method of claim 21 wherein a quinolone antibiotic is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein a quinolone antibiotic is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

CW211P

Abstract

A composition comprising a polypeptide and a quinolone antibiotic covalently attached to the polypeptide. Also provided is a method for delivery of a quinolone antibiotic to a patient comprising administering to the patient a composition comprising a polypeptide and a quinolone antibiotic covalently attached to the polypeptide. Also provided is a method for protecting a quinolone antibiotic from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of a quinolone antibiotic from a composition comprising covalently attaching it to the polypeptide.

CW212P

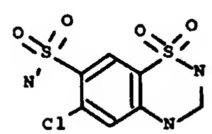
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING HYDROCHLOROTHIAZIDE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to hydrochlorothiazide, as well as methods for protecting and administering hydrochlorothiazide. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Hydrochlorothiazide is a known pharmaceutical agent that is used in the treatment of hypertension. The chemical structure of hydrochlorothiazide is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage

reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in

the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (hydrochlorothiazide) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching hydrochlorothiazide to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising hydrochlorothiazide microencapsulated by a polypeptide.

The invention provides a composition comprising a polypeptide and hydrochlorothiazide covalently attached to the polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Hydrochlorothiazide preferably is covalently attached to a side chain, the N-terminus or the C-terminus of the polypeptide. In a preferred embodiment, the active

agent is a carboxylic acid and is covalently attached to the N-terminus of the polypeptide. In another preferred embodiment, the active agent is an amine and is covalently attached to the C-terminus of the polypeptide. In another preferred embodiment, the active agent is an alcohol and is covalently attached to the C-terminus of the polypeptide. In yet another preferred embodiment, the active agent is an alcohol and is covalently attached to the N-terminus of the polypeptide.

The composition of the invention can also include one or more of a microencapsulating agent, an adjuvant and a pharmaceutically acceptable excipient. The microencapsulating agent can be selected from polyethylene glycol (PEG), an amino acid, a sugar and a salt. When an adjuvant is included in the composition, the adjuvant preferably activates an intestinal transporter.

Preferably, the composition of the invention is in the form of an ingestable tablet, an intravenous preparation or an oral suspension. The active agent can be conformationally protected by folding of the polypeptide about the active agent. In another embodiment, the polypeptide is capable of releasing the active agent from the composition in a pH-dependent manner.

The invention also provides a method for protecting hydrochlorothiazide from degradation comprising covalently attaching it to a polypeptide.

The invention also provides a method for delivering hydrochlorothiazide to a patient, the patient being a human or a non-human animal, comprising administering to the patient a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. In a preferred embodiment, hydrochlorothiazide is released from the composition by an enzyme-catalyzed release. In another preferred embodiment, hydrochlorothiazide is released in a time-dependent manner based on the pharmacokinetics of the enzyme-catalyzed release. In another preferred embodiment, the composition further comprises a microencapsulating agent and hydrochlorothiazide is released from the composition by dissolution of the microencapsulating agent. In another preferred embodiment, hydrochlorothiazide is released from the composition by a pH-dependent unfolding of the polypeptide. In another preferred embodiment,

hydrochlorothiazide is released from the composition in a sustained release. In yet another preferred embodiment, the composition further comprises an adjuvant covalently attached to the polypeptide and release of the adjuvant from the composition is controlled by the polypeptide. The adjuvant can be microencapsulated into a carrier peptide-drug conjugate for biphasic release of active ingredients.

The invention also provides a method for preparing a composition comprising a polypeptide and an active agent covalently attached to the polypeptide. The method comprises the steps of:

(a) attaching hydrochlorothiazide to a side chain of an amino acid to form an active agent/amino acid complex;

(b) forming an active agent/amino acid complex N-carboxyanhydride (NCA) from the active agent/amino acid complex; and

(c) polymerizing the active agent/amino acid complex N-carboxyanhydride (NCA).

In a preferred embodiment, steps (a) and (b) are repeated prior to step (c) with a second active agent. When steps (a) and (b) are repeated prior to step (c) with a second agent, hydrochlorothiazide and a second active agent can be copolymerized in step (c). In another preferred embodiment, the amino acid is glutamic acid and the active agent is released from the glutamic acid as a dimer upon a hydrolysis of the polypeptide and wherein the active agent is released from the glutamic acid by coincident intramolecular transamination. In another preferred embodiment, the glutamic acid is replaced by an amino acid selected from the group consisting of aspartic acid, arginine, asparagine, cysteine, lysine, threonine, and serine, and wherein the active agent is attached to the side chain of the amino acid to form an amide, a thioester, an ester, an ether, a urethane, a carbonate, an anhydride or a carbamate. In yet another preferred embodiment, the glutamic acid is replaced by a synthetic amino acid with a pendant group comprising an amine, an alcohol, a sulfhydryl, an amide, a urea, or an acid functionality.

It is to be understood that both the foregoing general description and the following detailed description are exemplary, but are not restrictive, of the invention. The general applications of this invention to other active pharmaceutical agents is

described in U.S. Patent Application Serial Number 09/642,820, filed August 22, 2000, incorporated herein by reference.

DETAILED DESCRIPTION OF INVENTION

The present invention provides several benefits for active agent delivery. First, the invention can stabilize hydrochlorothiazide and prevent its digestion in the stomach. In addition, the pharmacologic effect can be prolonged by delayed release of hydrochlorothiazide. Furthermore, active agents can be combined to produce synergistic effects. Also, absorption of the active agent in the intestinal tract can be enhanced. The invention also allows targeted delivery of active agents to specifics sites of action.

The composition of the invention comprises hydrochlorothiazide covalently attached to a polypeptide. Preferably, the polypeptide is (i) an oligopeptide, (ii) a homopolymer of one of the twenty naturally occurring amino acids, (iii) a heteropolymer of two or more naturally occurring amino acids, (iv) a homopolymer of a synthetic amino acid, (v) a heteropolymer of two or more synthetic amino acids or (vi) a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

Proteins, oligopeptides and polypeptides are polymers of amino acids that have primary, secondary and tertiary structures. The secondary structure of the protein is the local conformation of the polypeptide chain and consists of helices, pleated sheets and turns. The protein's amino acid sequence and the structural constraints on the conformations of the chain determine the spatial arrangement of the molecule. The folding of the secondary structure and the spatial arrangement of the side chains constitute the tertiary structure.

Proteins fold because of the dynamics associated between neighboring atoms on the protein and solvent molecules. The thermodynamics of protein folding and unfolding are defined by the free energy of a particular condition of the protein that relies on a particular model. The process of protein folding involves, amongst other things, amino acid residues packing into a hydrophobic core. The amino acid side chains inside the protein core occupy the same volume as they do in amino acid crystals. The folded

protein interior is therefore more like a crystalline solid than an oil drop and so the best model for determining forces contributing to protein stability is the solid reference state.

The major forces contributing to the thermodynamics of protein folding are Van der Waals interactions, hydrogen bonds, electrostatic interactions, configurational entropy and the hydrophobic effect. Considering protein stability, the hydrophobic effect refers to the energetic consequences of removing apolar groups from the protein interior and exposing them to water. Comparing the energy of amino acid hydrolysis with protein unfolding in the solid reference state, the hydrophobic effect is the dominant force. Hydrogen bonds are established during the protein fold process and intramolecular bonds are formed at the expense of hydrogen bonds with water. Water molecules are "pushed out" of the packed, hydrophobic protein core. All of these forces combine and contribute to the overall stability of the folded protein where the degree to which ideal packing occurs determines the degree of relative stability of the protein. The result of maximum packing is to produce a center of residues or hydrophobic core that has maximum shielding from solvent.

Since it is likely that lipophilic drugs would reside in the hydrophobic core of a peptide, it would require energy to unfold the peptide before the drug can be released. The unfolding process requires overcoming the hydrophobic effect by hydrating the amino acids or achieving the melting temperature of the protein. The heat of hydration is a destabilization of a protein. Typically, the folded state of a protein is favored by only 5-15 kcal/mole over the unfolded state. Nonetheless, protein unfolding at neutral pH and at room temperature requires chemical reagents. In fact, partial unfolding of a protein is often observed prior to the onset of irreversible chemical or conformation processes. Moreover, protein conformation generally controls the rate and extent of deleterious chemical reactions.

Conformational protection of active agents by proteins depends on the stability of the protein's folded state and the thermodynamics associated with the agent's decomposition. Conditions necessary for the agent's decomposition should be different than for protein unfolding.

Selection of the amino acids will depend on the physical properties desired. For instance, if increase in bulk or lipophilicity is desired, then the carrier polypeptide will be enriched in the amino acids in the table provided below. Polar amino acids, on the other hand, can be selected to increase the hydrophilicity of the polypeptide.

Ionizing amino acids can be selected for pH controlled peptide unfolding. Aspartic acid, glutamic acid and tyrosine carry a neutral charge in the stomach, but will ionize upon entry into the intestine. Conversely, basic amino acids, such as histidine, lysine and arginine, ionize in the stomach and are neutral in an alkaline environment.

Other factors such as π-π interactions between aromatic residues, kinking of the peptide chain by addition of proline, disulfide crosslinking and hydrogen bonding can all be used to select the optimum amino acid sequence for a given application. Ordering of the linear sequence can influence how these interactions can be maximized and is important in directing the secondary and tertiary structures of the polypeptide.

Furthermore, amino acids with reactive side chains (e.g., glutamic acid, lysine, aspartic acid, serine, threonine and cysteine) can be incorporated for attaching multiple active agents or adjuvants to the same carrier peptide. This is particularly useful if a synergistic effect between two or more active agents is desired.

As stated above, variable molecular weights of the carrier compound can have profound effects on the active agent release kinetics. As a result, low molecular weight active agent delivery systems are preferred. An advantage of this invention is that chain length and molecular weight of the polypeptide can be optimized depending on the level of conformational protection desired. This property can be optimized in concert with the kinetics of the first order release mechanism. Thus, another advantage of this invention is that prolonged release time can be imparted by increasing the molecular weight of the carrier polypeptide. Another, significant advantage of the invention is that the kinetics of active agent release is primarily controlled by the enzymatic hydrolysis of the key bond between the carrier peptide and the active agent.

Dextran is the only polysaccharide known that has been explored as a macromolecular carrier for the covalent binding of drug for colon specific drug delivery. Generally, it was only possible to load up to 1/10 of the total drug-dextran conjugate weight with drug. As stated earlier, polysaccharides are digested mainly in the colon and drug absorption is mainly limited to the colon. As compared to dextran, this invention has two major advantages. First, peptides are hydrolyzed by any one of several aminopeptidases found in the intestinal lumen or associated with the brush-border membrane and so active agent release and subsequent absorption can occur in the jejunum or the ileum. Second, the molecular weight of the carrier molecule can be controlled and, thus, active agent loading can also be controlled.

As a practical example, the following table lists the molecular weights of lipophilic amino acids (less one water molecule) and selected analgesics and vitamins.

TABLE

Amino acid	MW	Active agent	MW
Glycine	57	Acetaminophen	151
Alanine	71	Vitamin B_6 (Pyroxidine)	169
Valine	99	Vitamin C (Ascorbic acid)	176
Leucine	113	Aspirin	180
Isoleucine	113	Ibuprofen	206
Phenylalanine	147	Retinoic acid	300
Tyrosine	163	Vitamin B_2 (Riboflavin)	376
		Vitamin D_2	397
		Vitamin E (Tocopherol)	431

Lipophilic amino acids are preferred because conformational protection through the stomach is important for the selected active agents, which were selected based on ease of covalent attachment to an oligopeptide. Eighteen was subtracted from the amino acid's molecular weight so that their condensation into a polypeptide is considered. For example, a decamer of glycine (MW=588) linked to aspirin would have a total molecular weight of 750 and aspirin would represent 24% of the total weight of the active agent delivery composition or over two times the maximum drug loading for dextran. This is only for an N- or C- terminus application, for those active agents attached to pendant

groups of decaglutamic acid, for instance, a drug with a molecular weight of 180 could conceivably have a loading of 58%, although this may not be entirely practical.

The alcohol, amine or carboxylic acid group of an active agent may be covalently attached to the N-terminus, the C-terminus or the side chain of the oligopeptide or polypeptide. The location of attachment depends somewhat on the functional group selection. For instance, if the active drug is a carboxylic acid (e.g., aspirin) then the N-terminus of the oligopeptide is the preferred point of attachment. If the active agent is an amine (e.g., ampicillin), then the C-terminus is the preferred point of attachment in order to achieve a stable peptide linked active agent. In both, the C- and N-terminus examples, the peptide is, in essence, extended by one monomeric unit forming a new peptide bond. If the active agent is an alcohol, then either the C-terminus or the N-terminus is the preferred point of attachment in order to achieve a stable composition. As in the example above where the alcohol, norethindrone, was covalently attached to poly(hydroxypropylglutamine), an alcohol can be converted into an alkylchloroformate with phosgene. This invention, then, pertains to the reaction of this key intermediate with the N-terminus of the peptide carrier. The active ingredient can be released from the peptide carrier by intestinal peptidases.

The alcohol can be selectively bound to the gamma carboxylate of glutamic acid and then this conjugate covalently attached to the C-terminus of the peptide carrier. Because the glutamic acid-drug conjugate can be considered a dimer, this product adds two monomeric units to the C-terminus of the peptide carrier where the glutamic acid moiety serves as a spacer between the peptide and the drug as shown in Fig. 4. Intestinal enzymatic hydrolysis of the key peptide bond releases the glutamic acid-drug moiety from the peptide carrier. The newly formed free amine of the glutamic acid residue will then undergo an intramolecular transamination reaction, thereby, releasing the active agent with coincident formation of pyroglutamic acid as shown in Fig. 5. Alternatively, the glutamic acid-drug dimer can be converted into the gamma ester of glutamic acid N-carboxyanhydride. This intermediate can then be polymerized, as described above, using any suitable initiator as shown in Fig. 4. The product of this polymerization is polyglutamic acid with active ingredients attached to multiple pendant groups. Hence,

maximum drug loading of the carrier peptide can be achieved. In addition, other amino acid-NCA's can be copolymerized with the gamma ester glutamic acid NCA to impart specific properties to the drug delivery system.

The invention also provides a method of imparting the same mechanism of action for other polypeptides containing functional side chains. Examples include, but are not limited to, polylysine, polyasparagine, polyarginine, polyserine, polycysteine, polytyrosine, polythreonine and polyglutamine. The mechanism can translate to these polypeptides through a spacer or linker on the pendant group, which is terminated, preferably, by the glutamic acid-drug dimer. This carrier peptide-drug conjugate is distinguished from the prior art by virtue of the fact that the primary release of the drug moiety relies on peptidases and not on esterases. Alternatively, the active agent can be attached directly to the pendant group where some other indigenous enzymes in the alimentary tract can affect release.

The active agent can be covalently attached to the N-terminus, the C-terminus or the side chain of the polypeptide using known techniques. Examples of linking organic compounds to the N-terminus type of a peptide include, but are not limited to, the attachment of naphthylacetic acid to LH-RH, coumarinic acid to opioid peptides and 1,3-dialkyl-3-acyltriazenes to tetragastrin and pentagastrin. As another example, there are known techniques for forming peptide linked biotin and peptide linked acridine.

In the present invention, hydrochlorothiazide is covalently attached to the polypeptide via the amine group.

The polypeptide carrier can be prepared using conventional techniques. A preferred technique is copolymerization of mixtures of amino acid N-carboxyanhydrides. Alternatively, if a specific sequence is desired, a solid state automated peptide synthesizer can be used.

The addition of stabilizers to the composition has the potential of stabilizing the polypeptide further. Stabilizers such as sugar, amino acids, polyethylene glycol (PEG) and salts have been shown to prevent protein unfolding. In another embodiment of the

invention, a pre-first order release of the active agent is imparted by microencapsulating the carrier polypeptide-active agent conjugate in a polysaccharide, amino acid complex, PEG or salts.

There is evidence that hydrophilic compounds are absorbed through the intestinal epithelia efficiently via specialized transporters. The entire membrane transport system is intrinsically asymmetric and responds asymmetrically to cofactors. Thus, one can expect that excitation of the membrane transport system will involve some sort of specialized adjuvant resulting in localized delivery of active agents. There are seven known intestinal transport systems classified according to the physical properties of the transported substrate. They include the amino acid, oligopeptide, glucose, monocarboxic acid, phosphate, bile acid and the P-glycoprotein transport systems and each has its own associated mechanism of transport. The mechanisms can depend on hydrogen ions, sodium ions, binding sites or other cofactors. The invention also allows targeting the mechanisms for intestinal epithelial transport systems to facilitate absorption of active agents.

In another embodiment of the invention, the composition includes one or more adjuvants to enhance the bioavailability of the active agent. Addition of an adjuvant is particularly preferred when using an otherwise poorly absorbed active agent. Suitable adjuvants, for example, include: papain, which is a potent enzyme for releasing the catalytic domain of aminopeptidase-N into the lumen; glycorecognizers, which activate enzymes in the BBM; and bile acids, which have been attached to peptides to enhance absorption of the peptides.

Preferably, the resultant peptide-hydrochlorothiazide conjugate is formulated into a tablet using suitable excipients and can either be wet granulated or dry compressed.

Compositions of the invention are, in essence, the formation of amides from acids and amines and can be prepared by the following examples.

Acid/N-terminus conjugation

An acid bioactive agent can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine peptide carrier. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, the product precipitated out in ether and purified using gel permeation chromatography (GPC) or dialysis.

Amine/C-terminus conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the amine bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Alcohol/N-Terminus Conjugation

In the following example the combination of the alcohol with triphosgene produces a chloroformate, which when reacted with the N-terminus of the peptide produces a carbamate. Pursuant to this, an alcohol bioactive agent can be treated with triphosgene in dry DMF under nitrogen. The suitably protected peptide carrier is then added slowly and the solution stirred at room temperature for several hours. The product is then precipitated out in ether. The crude product is suitably deprotected and purified using GPC.

Other solvents, activating agents, cocatalysts and bases can be used. Examples of other solvents include dimethylsulfoxide, ethers such as tetrahydrofuran or chlorinated solvents such as chloroform. Examples of other activating agents include dicyclohexylcarbodiimide or thionyl chloride. An example of another cocatalyst is N-hydroxysuccinimide. Examples of bases include pyrrolidinopyridine, dimethylaminopyridine, triethylamine or tributylamine.

Preparation of γ-Alkyl Glutamate

There have been over 30 different γ-alkyl glutamates prepared any one of which may be suitable for the drug alcohol of choice. For example, a suspension of glutamic acid, the alcohol and concentrated hydrochloric acid can be prepared and heated for several hours. The γ-alkyl glutamate product can be precipitated out in acetone, filtered, dried and recrystallized from hot water.

γ-Alkyl Glutamate/C-Terminus Conjugation

The peptide carrier can be dissolved in DMF under nitrogen and cooled to $0^{O}C$. The solution can then be treated with diisopropylcarbodiimide and hydroxybenzotriazole followed by the γ-alkyl glutamate bioactive agent. The reaction can then be stirred for several hours at room temperature, the urea by-product filtered off, and the product precipitated out in ether and purified using GPC or dialysis.

Preparation of γ-Alkyl Glutamate-NCA

γ-Alkyl glutamate can be suspended in dry THF where triphosgene is added and the mixture refluxed under a nitrogen atmosphere until the mixture becomes homogenous. The solution can be poured into heptane to precipitate the NCA product, which is filtered, dried and recrystallized from a suitable solvent.

Preparation of Poly[γ-Alkyl Glutamate]

γ-Alkyl glutamate-NCA can be dissolved in dry DMF where a catalytic amount of a primary amine can be added to the solution until it becomes viscous (typically overnight). The product can be isolated from the solution by pouring it into water and filtering. The product can be purified using GPC or dialysis.

Although illustrated and described above with reference to specific embodiments, the invention is nevertheless not intended to be limited to the details shown. Rather, various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
a polypeptide; and
hydrochlorothiazide covalently attached to said polypeptide.

2. The composition of claim 1 wherein said polypeptide is an oligopeptide.

3. The composition of claim 1 wherein said polypeptide is a homopolymer of a naturally occurring amino acid.

4. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more naturally occurring amino acids.

5. The composition of claim 1 wherein said polypeptide is a homopolymer of a synthetic amino acid.

6. The composition of claim 1 wherein said polypeptide is a heteropolymer of two or more synthetic amino acids.

7. The composition of claim 1 wherein said polypeptide is a heteropolymer of one or more naturally occurring amino acids and one or more synthetic amino acids.

8. The composition of claim 1 wherein hydrochlorothiazide is covalently attached to a side chain, the N-terminus or the C-terminus of said polypeptide.

9. The composition of claim 1 further comprising a microencapsulating agent.

10. The composition of claim 9 wherein said microencapsulating agent is selected from the group consisting of polyethylene glycol (PEG), an amino acid, a sugar and a salt.

11. The composition of claim 1 further comprising an adjuvant.

12. The composition of claim 11 wherein said adjuvant activates an intestinal transporter.

13. The composition of claim 1 further comprising a pharmaceutically acceptable excipient.

14. The composition of claim 1 wherein said composition is in the form of an ingestable tablet.

15. The composition of claim 1 wherein said composition is in the form of an intravenous preparation.

16. The composition of claim 1 wherein said composition is in the form of an oral suspension.

17. The composition of claim 1 wherein hydrochlorothiazide is conformationally protected by folding of said polypeptide about said active agent.

18. The composition of claim 1 wherein said polypeptide is capable of releasing hydrochlorothiazide from said composition in a pH-dependent manner.

19. A method for protecting hydrochlorothiazide from degradation comprising covalently attaching said active agent to a polypeptide.

20. A method for controlling release of hydrochlorothiazide from a composition wherein said composition comprises a polypeptide, said method comprising covalently attaching hydrochlorothiazide to said polypeptide.

21. A method for delivering hydrochlorothiazide to a patient comprising administering to said patient a composition comprising:

a polypeptide; and

hydrochlorothiazide covalently attached to said polypeptide.

22. The method of claim 21 wherein hydrochlorothiazide is released from said composition by an enzyme-catalyzed release.

23. The method of claim 21 wherein hydrochlorothiazide is released from said composition by a pH-dependent unfolding of said polypeptide.

24. The method of claim 21 wherein said active agent is released from said composition in a sustained release.

25. The method of claim 21 wherein said composition further comprises an adjuvant covalently attached to said polypeptide and wherein release of said adjuvant from said composition is controlled by said polypeptide.

Abstract

A composition comprising a polypeptide and hydrochlorothiazide covalently attached to the polypeptide. Also provided is a method for delivery of hydrochlorothiazide to a patient comprising administering to the patient a composition comprising a polypeptide and hydrochlorothiazide covalently attached to the polypeptide. Also provided is a method for protecting hydrochlorothiazide from degradation comprising covalently attaching it to a polypeptide. Also provided is a method for controlling release of hydrochlorothiazide from a composition comprising covalently attaching it to the polypeptide.

CW213P

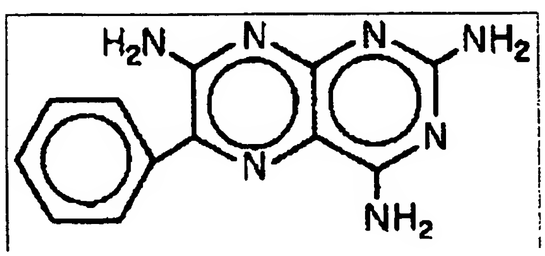
A NOVEL PHARMACEUTICAL COMPOUND CONTAINING HYDROCHLOROTHIAZIDE AND TRIAMTERENE AND METHODS OF MAKING AND USING SAME

FIELD OF THE INVENTION

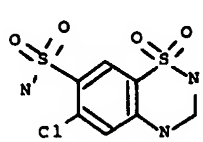
The present invention relates to a novel pharmaceutical compound that comprises a polypeptide that is preferably covalently attached to hydrochlorothiazide and triamterene, as well as methods for protecting and administering hydrochlorothiazide and triamterene. This novel compound, referred to as a CARRIERWAVE™ Molecular Analogue (CMA), has the benefit of taking a known effective pharmaceutical agent that is both well studied and occupies a known segment of the pharmaceutical market, and combining it with a carrier compound that enhances the usefulness of the pharmaceutical agent without compromising its pharmaceutical effectiveness.

BACKGROUND OF THE INVENTION

Hydrochlorothiazide and triamterene are known pharmaceutical agents that are used together in the treatment of edema and hypertension. The chemical structure of triamterene is:



Its chemical name is -----. The chemical structure of hydrochlorothiazide is:



The novel pharmaceutical compound of the present invention is useful in accomplishing one or more of the following goals: enhancement of the chemical stability of the original compound; alteration of the release profile of an orally administered product; enhanced digestion or absorption; targeted delivery to particular tissue/cell type; and provision for an oral dosage form when none exists. The novel pharmaceutical compound may contain one or more of the following: another active pharmaceutical agent, an adjuvant, or an inhibitor.

Active agent delivery systems are often critical for the effective delivery of a biologically active agent (active agent) to the appropriate target. The importance of these systems becomes magnified when patient compliance and active agent stability are taken under consideration. For instance, one would expect patient compliance to increase markedly if an active agent is administered orally in lieu of an injection or another invasive technique. Increasing the stability of the active agent, such as prolonging shelf life or survival in the stomach, will assure dosage reproducibility and perhaps even reduce the number of dosages required which could improve patient compliance.

Absorption of an orally administered active agent is often blocked by the harshly acidic stomach milieu, powerful digestive enzymes in the GI tract, permeability of cellular membranes and transport across lipid bilayers. Incorporating adjuvants such as resorcinol, surfactants, polyethylene glycol (PEG) or bile acids enhance permeability of cellular membranes. Microencapsulating active agents using protenoid microspheres, liposomes or polysaccharides have been effective in abating enzyme degradation of the active agent. Enzyme inhibiting adjuvants have also been used to prevent enzyme degradation. Enteric coatings have been used as a protector of pharmaceuticals in the stomach.

Active agent delivery systems also provide the ability to control the release of the active agent. For example, formulating diazepam with a copolymer of glutamic acid and aspartic acid enables a sustained release of the active agent. As another example, copolymers of lactic acid and glutaric acid are used to provide timed release of human

growth hormone. A wide range of pharmaceuticals purportedly provide sustained release through microencapsulation of the active agent in amides of dicarboxylic acids, modified amino acids or thermally condensed amino acids. Slow release rendering additives can also be intermixed with a large array of active agents in tablet formulations.

Each of these technologies imparts enhanced stability and time-release properties to active agent substances. Unfortunately, these technologies suffer from several shortcomings. Incorporation of the active agent is often dependent on diffusion into the microencapsulating matrix, which may not be quantitative and may complicate dosage reproducibility. In addition, encapsulated drugs rely on diffusion out of the matrix, which is highly dependant on the water solubility of the active agent. Conversely, water-soluble microspheres swell by an infinite degree and, unfortunately, may release the active agent in bursts with little active agent available for sustained release. Furthermore, in some technologies, control of the degradation process required for active agent release is unreliable. For example, an enterically coated active agent depends on pH to release the active agent and, as such, is difficult to control the rate of release.

In the past, use has been made of amino acid side chains of polypeptides as pendant groups to which active agents can be attached. These technologies typically require the use of spacer groups between the amino acid pendant group and the active agent. The peptide-drug conjugates of this class of drug delivery system rely on enzymes in the bloodstream for the release of the drug and, as such, are not used for oral administration. Examples of timed and targeted release of injectable or subcutaneous pharmaceuticals include: linking of norethindrone, via a hydroxypropyl spacer, to the gamma carboxylate of polyglutamic acid; and linking of nitrogen mustard, via a peptide spacer, to the gamma carbamide of polyglutamine. Dexamethasone has been covalently attached directly to the beta carboxylate of polyaspartic acid without a spacer group. This prodrug formulation was designed as a colon-specific drug delivery system where the drug is released by bacterial hydrolytic enzymes residing in the large intestines. The released dexamethasone active agent, in turn, was targeted to treat large bowel disorders and was not intended to be absorbed into the bloodstream. Yet another technology combines the advantages of covalent drug attachment with liposome formation where the

active ingredient is attached to highly ordered lipid films (known as HARs) via a peptide linker. Thus, there has been no drug delivery system, heretofore reported, that incorporates the concept of attaching an active ingredient to a polypeptide pendant group with its targeted delivery into the bloodstream via oral administration.

It is also important to control the molecular weight, molecular size and particle size of the active agent delivery system. Variable molecular weights have unpredictable diffusion rates and pharmacokinetics. High molecular weight carriers are digested slowly or late, as in the case of naproxen-linked dextran, which is digested almost exclusively in the colon by bacterial enzymes. High molecular weight microspheres usually have high moisture content which may present a problem with water labile active ingredients. Particle size not only becomes a problem with injectable drugs, as in the HAR application, but absorption through the brush-border membrane of the intestines is limited to less than 5 microns.

SUMMARY OF THE INVENTION

The present invention provides covalent attachment of the active agent (hydrochlorothiazide and triamterene) to a polymer of peptides or amino acids. The invention is distinguished from the above-mentioned technologies by virtue of covalently attaching hydrochlorothiazide and triamterene to the N-terminus, the C-terminus or directly to the amino acid side chain of an oligopeptide or polypeptide, also referred to herein as a carrier peptide. In certain applications, the polypeptide will stabilize the active agent, primarily in the stomach, through conformational protection. In these applications, delivery of the active agent is controlled, in part, by the kinetics of unfolding of the carrier peptide. Upon entry into the upper intestinal tract, indigenous enzymes release the active ingredient for absorption by the body by selectively hydrolyzing the peptide bonds of the carrier peptide. This enzymatic action introduces a second order sustained release mechanism.

Alternatively, the present invention provides a pharmaceutical composition comprising hydrochlorothiazide and triamterene microencapsulated by a polypeptide.